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On the Relation of Gastroptosis to Blood-diastase, -sugar and -glycogen

By

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Gastroptosis is characterized by a lengthweise extension of the stomach such that the greater curvature is not held on the level of the navel but reaches down into the little pelvic cavity. When the tension of the stomach relaxes, the stomach shows a state of atonic gastroptosis, and the external aspect of the patients is often called Habitus atonicus. Such cases may well be accompanied by low blood-pressure, the question of primary or secondary genesis in this complication being unimportant.

From this point of view, the changes of blood-diastase, -sugar and -glycogen, which are closely connected with digestion or absorption, have been investigated in the gastroptotic patients, and then the relations or multiplied relations between gastroptosis and low blood-pressure observed.

Experimental

Methods

In the case of gastroptosis the X-ray image of the lesser curvature-edge always appears under the level of the navel or iliac crest at any possible phase of stomach peristalsis when the stomach is given about 400 cc. contrast media.

It this study, when the maximum blood-pressure runs constantly higher than 150 mm. Hg, it is called hypertension; and when the maximum blood-pressure usually runs lower than 110 mm. Hg, it is called hypotension.

The subjects were 75 persons, from 15 to 66 years of age (an average of 33.5 years), selected from out- and in-patients with internal ailments, from members of our hospital, and from girls of our nursing school.

The blood-diastase was estimated by Baltzer's method,[1] the blood-sugar by Hagedorn-Jensen's method and the blood-glycogen by the Golanda's method.[2] In these examinations the blood was taken with the patient in hungry condition before breakfast and usually from an anticubital vein, and was immediately mixed with 2 mg. sodium citrate

per 1 cc. blood in order to prevent coagulation of the blood. The gastric juice was drawn out with the duodenal tube at regular intervals, and its acidity estimated.

Results

The blood-diastase, -sugar and -glycogen values of the control group

Prior to this experiment a number of healthy persons and others whose stomachs were in normal condition but who exhibited hyper- or hypotension were selected as controls. Of course, the persons of the latter group were supposed not to have suffered from any liver, kidney, or lung disease etc. The control group of 24 cases consisted of 12 males and 12 females and was divided into three type-groups, 6 cases with low, 13 with normal and 5 with high blood pressure.

Normal stomach and normal blood pressure (13 *cases*)

With the exception of No. 3, the blood-diastase values in all cases ranked from 177 to 401 mg/dl and the average value was 243 mg/dl. These values approximated the range shown in Yamagata's work[3] i.e. from 166 to 429 mg/dl. The blood-sugar values were normal, i.e. from 81 to 101 mg/dl. The blood-glycogen values lay between 8.3 and 13.2 mg/dl, which accorded approximately with the normal range of 8.6 to 13.3 mg/dl previously reported by Sikinami and his co-workers[4].

Normal stomach with hypotension (6 cases)

The blood-diastase values were normal, i.e. between 243 and 422 mg/dl; the blood-sugar values were also normal, i.e. from 84 to 120 mg/dl; and the blood-glycogen values, ranging from 8.3 to 11.6 mg/dl, were also normal.

Normal stomach with hypertension (5 *cases*)

The blood-diastase values were normal, i.e. from 259 to 433 mg/dl. Except for No. 20, the blood-sugar values were normal, i.e. from 88 to 104 mg/dl. The blood-glycogen value in No. 23 was a bit high, but in the other 4 cases these values were normal, ranging from 9.5 to 10.7 mg/dl.

From these control experiments it may be concluded that the blood-diastase, -sugar and -glycogen values in patients with no stomach disease are normal regardless of differences in blood-pressure.

These Results are shown in Table I.

The blood-diastase, -sugar and -glycogen values of gastroptotic patients

The examined gastroptotic patients numbered 51 in all; 7 cases with normal blood pressure, 36 cases with low, and 8 cases with high. There were 12 males and 39 females.

Gastroptosis with normal blood-pressure (7 *cases*)

Except for No. 29, all cases exhibited the normal blood-diastase values of 196 to 324 mg/dl. The blood-sugar values also showed the normal

TABLE I

Experimental Results of Normal Stomach Cases

Number	Subject			Blood-pressure (mm.Hg.)	Blood		
	Name	Sex	Age		Diastase (mg/dl)	Sugar (mg/dl)	Glycogen (mg/dl)
				1) Normal blood pressure			
1	S.M.	♀	21	120- 60	296	85	13.2
2	I.K.	♀	26	120- 70	372	92	10.7
3	H.A.	♀	19	120- 70	551	83	11.0
4	S.S.	♂	29	130- 60	306	88	10.2
5	R.K.	♂	30	130- 70	401	90	8.5
6	M.F.	♂	21	120- 70	222	93	11.9
7	S.T.	♂	51	130- 80	315	81	10.4
8	N.N.	♂	33	120- 70	376	101	9.2
9	K.S.	♀	23	120- 60	323	86	10.3
10	R.N.	♀	22	120- 70	179	88	8.3
11	T.I.	♂	29	120- 60	216	86	8.8
12	T.K.	♀	19	120- 40	177	90	8.5
13	G.S.	♂	31	130- 60	217	90	8.5
	Mean				283	89	10.0
	Range				177-401 with exception of No. 3	81-101	8.3-11.9
				2) Low blood pressure			
14	M.S.	♀	19	110- 70	376	84	8.3
15	T.I.	♀	17	110- 70	278	84	9.8
16	K.M.	♀	19	110- 68	367	101	11.6
17	T.W.	♀	16	110- 70	243	120	11.1
18	Y.S.	♀	17	110- 50	390	86	8.3
19	R.H.	♂	48	110- 54	442	84	9.6
	Mean				349	93	9.8
	Range				243-442	84-120	8.3-11.6
				3) High blood pressure			
20	T.T.	♀	57	170-100	380	70	9.5
21	T.K.	♂	19	160- 90	318	88	10.7
22	K.S.	♂	28	170- 80	328	90	9.5
23	S.S.	♂	38	180- 80	259	99	15.9
24	G.I.	♂	47	170-100	433	104	9.8
	Mean				344	89	11.1
	Range				259-433	70-104	9.5-15.9

range of 79 to 90 mg/dl. In regard to blood-glycogen values, 4 cases (Nos. 25, 26, 29 and 30) had the low values of 6.7, 7.5, 7.7 and 6.3 mg/dl respectively, but the other 3 cases gave the normal range of 8.3 to 10.0 mg/dl.

Gastroptosis with hypotension (36 *cases*)

The blood-diastase value of Nos. 32, 66 and 67 were 640, 518 and 499

TABLE II

Experimental Results of Gastroptotic Patients

Number	Subject			Gastro-ptosis	Gastric atonia	Blood-pressure	Blood			Free HCl of gastric juice	Appetite	Complication
	Name	Sex	Age				Diastase (mg/dl)	Sugar (mg/dl)	Glycogen (mg/dl)			
1) Normal blood pressure												
25	F.O.	♀	26	##	++	120- 70	283	90	6.3	Low	Poor	
26	S.K.	♀	29	+-	+	124- 70	306	92	7.5	Low	Poor	
27	K.O.	♀	19	+	+	120- 60	324	97	8.3	No	Normal	
28	T.F.	♂	25	+	±	120- 80	276	83	8.3	Normal	Poor	Early infiltration
29	S.U.	♂	27	##	++	130- 70	571	84	7.7	Excess	Normal	
30	T.I.	♀	23	##	++	130- 60	196	79	6.3	Excess	Poor	
31	S.A.	♀	25	++	+	125- 70	298	88	10.3	Excess	Poor	
				Mean			322	88	9.1			
				Range			196–571	79–97	6.3–10.3			
2) Low blood pressure												
32	K.K.	♀	65	++	+	110- 60	640	92	9.8	No	Poor	Aortitis
33	K.H.	♀	33	++	+	110- 50	300	88	9.2	Normal	Poor	
34	Y.S.	♂	33	+	±	110- 70	301	79	7.1	Excess	Poor	
35	A.H.	♀	30	++	+	110- 70	332	88	10.3	Excess	Poor	
36	F.M.	♀	66	+	+	110- 60	346	95	7.5	Low	Poor	
37	F.K.	♀	18	##	++	110- 60	398	84	8.3	Normal	Normal	Heart valve disease
38	T.K.	♂	46	##	++	110- 60	370	95	7.1	Low	Normal	
39	M.S.	♀	30	+	±	110- 50	405	99	7.0	Normal	Poor	
40	R.M.	♀	20	##	+	95- 60	298	81	5.8	Low	Poor	
41	K.S.	♀	40	+	±	110- 70	175	72	7.5	Normal	Normal	
42	Y.N.	♀	59	##	++	110- 60	319	90	8.2	Excess	Poor	
43	M.N.	♀	43	+	+	110- 60	446	81	9.8	Excess	Poor	
44	K.S.	♀	40	##	++	100- 60	321	84	9.4	Low	Normal	
45	N.T.	♀	64	##	++	110- 60	309	83	10.3	Low	Poor	
46	K.S.	♀	39	+	±	90- 60	316	77	6.1	Low	Poor	
47	M.T.	♂	48	++	+	110- 70	455	81	9.4	Normal	Poor	
48	Y.O.	♀	19	+	±	90- 60	380	83	7.4	Excess	Poor	

49	K.S.	♀	33	+	+	110- 70	220	81	8.5	Normal	Poor	
50	H.T.	♀	33	+	±	110- 60	451	84	6.7	Normal	Normal	
51	T.K.	♀	29	++	++	110- 60	409	81	7.8	Normal	Normal	
52	B.F.	♂	62	+	±	100- 65	435	95	8.3	Normal	Normal	
53	S.A.	♀	37	+	+	110- 70	260	84	9.4	Excess	Poor	
54	T.S.	♂	30	++	+	110- 60	331	93	9.4	Normal	Normal	
55	K.S.	♀	30	++	++	110- 70	343	86	6.9	Normal	Poor	
56	K.K.	♀	26	+	±	100- 60	400	83	9.4	Low	Poor	
57	K.K.	♀	27	+	+	106- 40	442	99	10.3	Low	Poor	
58	K.M.	♀	39	++	+	100- 60	442	88	9.8	Normal	Poor	
59	S.N.	♀	35	+	±	100- 60	284	81	5.6	Normal	Poor	Elapsed pleuritis
60	A.O.	♀	65	+	±	110- 60	298	84	7.9	Low	Poor	Bronchitis
61	K.S.	♀	29	+	+	110- 70	354	79	8.1	Excess	Poor	Laryngeal tuberculose
62	J.M.	♂	24	+	+	110- 70	342	93	9.8	Low	Poor	Perihilar infiltration
63	H.F.	♂	49	+	+	90- 40	383	92	8.8	Normal	Normal	
64	K.N.	♀	15	++	+	110- 70	364	75	8.0	Low	Poor	
65	Y.M.	♀	34	++	+	110- 70	302	88	9.2	Normal	Normal	Ovarium-hypofunction
66	T.Y.	♂	15	++	+	110- 60	518	80	6.8	Low	Poor	
67	M.I.	♀	34	+	+	110- 70	499	92	7.0	No	Normal	
				Mean			366	86	8.3			
				Range			175–640	72–99	5.6–10.3			

3) High blood pressure

68	T.K.	♀	26	++	++	150- 80	303	90	6.7	Excess	Poor	
69	F.S.	♀	28	+	+	160- 90	324	90	9.0	No	Poor	
70	M.M.	♀	58	+	±	160-100	302	92	10.8	Excess	Normal	Chronic gonitis
71	K.I.	♂	28	++	+	150- 70	293	84	11.0	Excess	Normal	Nephritis
72	N.W.	♀	36	++	+	150- 90	280	72	10.3	Normal	Normal	
73	F.N.	♀	35	++	+	150- 80	486	86	8.1	Normal	Normal	
74	K.S.	♂	27	++	+	150- 70	391	88	6.7	Normal	Normal	
75	H.M.	♀	48	+	+	160- 90	485	90	7.0	Normal	Normal	
				Mean			358	88	8.6			
				Range			280–485	72–92	6.7–11.0			

mg/dl respectively; but the other 33 cases showed the normal range of 175 to 455 mg/dl. The blood-sugar values of Nos. 41, 44 and 64 were as low as 72, 77 and 75 mg/dl: but the other 33 cases kept the normal values of 79 to 99 mg/dl. The blood-glycogen values of Nos. 40, 59 and 46 were remarkably low, i.e. 5.8, 5.6 and 6.1 mg/dl; and those of Nos. 34, 36, 38, 39, 41, 48, 50, 51, 55, 60, 66 and 67 proved to be 7.1, 7.5, 7.1, 7.0, 7.5, 7.4, 6.7, 7.8, 6.9, 7.9, 6.8 and 7.0 mg/dl, respectively. These also are regarded as low values. In other words, the blood-glycogen was found to fall in 15 cases out of 36.

Gastroptosis with hypertension (8 *cases*)

The blood-diastase values of Nos. 73 and 75 were high, (486 and 485 mg/dl), but the other 6 cases showed the normal values of 280 to 391 mg/dl. Except for No. 72, the blood-sugar values in all cases were normal, i.e. between 84 and 90 mg/dl. The blood-glycogen values of Nos. 68, 74 and 75 were as low as 6.7, 6.7 and 7.0 mg/dl, but those of the other 5 cases stayed in the normal range of 8.1 to 11.0 mg/dl.

In cases of gastroptosis, as mentioned above, any changes were not recognized in blood-diastase and -sugar values. However the blood-glycogen values decreased in 22 cases out of 51 (43.1%). The blood-glycogen decrease was found very markedly in cases of hypotension.

See Table II.

Acid levels of gastric juice and the blood-glycogen values in gastroptotic patients

The former gastric juice in the gastroptotic patients showed a tendency to no acidity or low acid content, but in the majority of these patients the acid values became normal after the patients were given caffein solution as a test drink.

With respect to the free hydrochloric acid values of the former gastric juice in gastroptotic patients, the hypotension group was lacking in this acid more frequently than the nonhypotension group. When the blood-glycogen values of gastroptotic hypotonic patients were separated in two groups, i.e. those with values equivalent to or more than 8.0 mg/dl and those with values equivalent to or less than 7.9 mg/dl, the cases lacking in hydrochloric acid were observed more markedly in the latter group than in the former.

Anorexia of gastroptotic patients was found in 7 out of 15 cases without hypotension (48.6%), but in 25 out of 36 cases with hypotension (69.3%), and the anorexia became severe in cases with blood-glycogen values equal to or less than 7.9 mg/dl.

Results are shown in Table III and Figure 1 and 2.

Discussion

According to Sikinami *et al.*[4),5)] the glycogen-value of ductus thoracicus

TABLE III

Free Hydrochloric Acid of Former Gastric Juice in Gastroptotic Patients

Gastroptosis \ Acid level	0	1~10	10~20	21~	Total
With low blood pressure	23	8	1	3	35
With normal et high blood pressure	6	3	2	1	12
Total	29	11	3	4	47

Fig. 1 Fig. 2

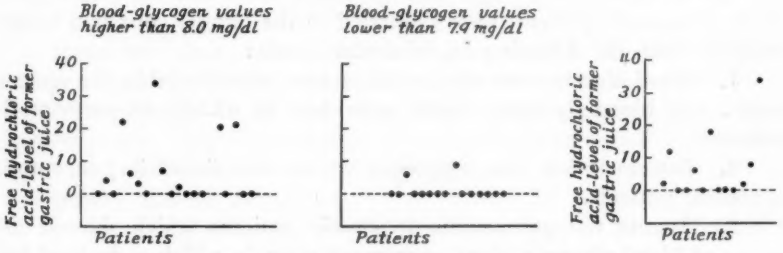

Fig. 1. Relations of blood-glycogen values to free hydrochloric acid-levels of former gastric juice in gastroptotic patients with low blood pressure.

Fig. 2. Free hydrochloric acid-levels of former gastric juice in gastroptotic patients without low blood pressure.

is always lower than that of venae hepaticae but at different times higher or lower than that of veins and arteries. The blood-glycogen value of the vein can never surpass that of the corresponding artery. Therefore it follows that the glycogen of ductus thoracicus must come from the blood vessel i.e. the liver capillary, and the blood-glycogen originates in the liver. The blood-glycogen values of healthy individuals are between 8.6 and 13.3 mg/dl, but in the cases of liver parenchym-damage[6] and diabetes mellitus[7),8)] they increase even in patients in the hungry condition.

The normal blood-diastase value ranges between 177 and 401 mg/dl, while, as already reported by Katsch[9)], it increases remarkably in the pancreas-ill cases. It is also observed in cases of liver parenchym-damage although not so strikingly as in pancreas-ill cases.[8),10),11),12)]

In this experiment with gastroptotic patients in the hungry condition before breakfast the blood-diastase and -sugar values were mostly normal.

This indicates that there was no damage in the pancreas. When there are no remarkable changes in the blood-diastase and -sugar values

but the blood-glycogen values are low, the conditions may be due to the decreased glycogen-supply from the liver to the blood vessel caused by the diminisched glycogen-depot of liver resulting from decline and delay in food digestion and absorption. This opinion was justified by the fact that the patients of blood-glycogen decreasing gastroptosis were lacking in free hydrochloric acid in their former gastric juice and were suffering from severe anorexia. Hence in these cases " Vagostigmin " was injected in order to increase the tension of the stomach etc., and sugar was administered for nutrition supply. This treatment has often proved effective in easing the clinical symptoms.

Conclusion

The blood-diastase, -sugar and -glycogen values and the gastric juice of 51 gastroptotic patients were estimated in the hungry condition before breakfast with the following experimental results:

1. Blood-diastase and -sugar values were mostly within the normal range, but blood-glycogen values were low in 43.1% of gastroptotic patients.
2. Extremely low blood-glycogen values were found in gastroptotic hypotonic patients.
3. Among the gastroptotic hypotonic patients which showed decrease of blood-glycogen, there were many cases in which a lack of free hydrochloric acid in former gastric juice together with anorexia was observed.

From these facts it has come to a conclusion that the blood-glycogen decrease of gastroptotic patients is possibly influenced by poor glycogen storage in liver which is caused by decline and delay in food digestion and absorption.

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Standard Sugar Loading Test

Special Reference to the Possibility of Differentiation of Two Types of Diabetes Mellitus and of Differentiation of Non-diabetics from Diabetics by Sugar Loading Test

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Many investigators have endeavoured to simplify the technique and interpretation of the sugar loading test. However, the literatures do not show agreement in the interpretation of an equivocal or transitional sugar loading curve. Misinterpretation of an equivocal sugar loading cuve may cause the patient to be denied life insurance or may cause him to undergo unnecessary diabetic treatment.

In the present study the standard sugar loading test was performed in healthy adults, in non-diabetic patients and in diabetic patients. Upon the basis of these results the differentiation of the transitional sugar loading curve from the diabetic curve is discussed. Furthermore, the possibility of determining the need for insulin in a given diabetic patient by means of the sugar loading test is described.

Experimental

Material and Techniques

Test subjects consisted of 17 healthy adults, 37 non-diabetic patients, 6 renal glycosuric patients and 76 diabetic patients. They were all hospitalized except the 17 normal adults. In this paper the diabetic patients are classified into two groups according to therapeutic requirements.[1] Type I diabetes mellitus includes patients who are controlled by our high carbohydrate and low fat diet alone, type II diabetes mellitus includes patients who require insulin as well as dietary control.

The sugar loading test was performed as follows. After an overnight fast, 50 g. of glucose dissolved in 250 cc. of water was administered orally. Blood specimens were collected from the ear lobe for blood sugar estimation prior to and every 30 minutes after the glucose administration for four hours. Urine specimens were collected every one hour. Blood sugar determinations were made by the Asari modification[2] of Fujita and Iwatake's micromethod[3]: and

urine sugar was estimated by the method of Bertrand.[4]

In diabetic patients the sugar loading test was performed within a week of hospitalization, prior to insulin treatment.

Result

Evaluation of the normal sugar loading curve

Healthy students and interns were chosen as a normal group. The results are summarized in Table I and Fig. 1. The mean fasting blood

TABLE I

Statistical Summary of Results of Oral Sugar Loading Test

The values in parentheses represent S.D. (Standard Deviation).

Group	Blood Sugar (mg. per cent)						
	Fasting	1 hr.	2 hr.	3hr.	4 hr.	Peak Level	Max. Increment of Blood Sugar
Healthy	107 (10)	141 (23)	98 (16)	86 (10)	90 (11)	159 (20)	52 (19)
Non-diabetic	110 (12)	153 (37)	106 (24)	94 (18)	90 (29)	165 (19)	55 (18)
Renal glycosuria	107 (8)	148 (33)	95 (12)	94 (12)	94 (3)	170 (19)	59 (22)
Type I diabetic	159 (36)	272 (44)	222 (58)	158 (55)	127 (37)	280 (52)	119 (34)
Type II diabetic	268 (50)	405 (66)	366 (68)	305 (69)	266 (63)	415 (67)	147 (29)

sugar was 107 mg.% with a S. D. (standard deviation) of 10 mg.%. The upper limit of normal for the fasting blood sugar in the healthy group was 117 mg.% when M+S.D. was used. 5 of the 17 cases exceeded this level, but none of them showed a fasting blood sugar value above 120 mg.% which is widely accepted as the upper limit of normal fasting blood sugar level.[5] The blood sugar curve of the healthy group rose rapidly and reached a peak level at $\frac{1}{2}$ or 1 hour and fell rapidly to hypoglycemic level. The mean value of the peak level was 159 mg.%; and the upper limit was 179 mg.% when M+S.D. was used. 3 cases exceeded this level; but all cases were included within 199 mg.% (M+2 S.D.). These results support the criterion that the normal alimentary hyperglycemia does not exceed 200 mg.% for capillary blood.[5] The maximum increment of blood sugar due to glucose ingestion was 52 mg.% (ranging 10 to 88 mg.%). Hypoglycemic phase began to occur usually at $1\frac{1}{2}$ or 2 hours; and the blood sugar returned to near the initial value at 3 or 4 hours. The secondary hyperglycemic rebound phenomenon was not observed. The mean 2 hour blood sugar level was 98 mg.% (73—134 mg.%). The upper

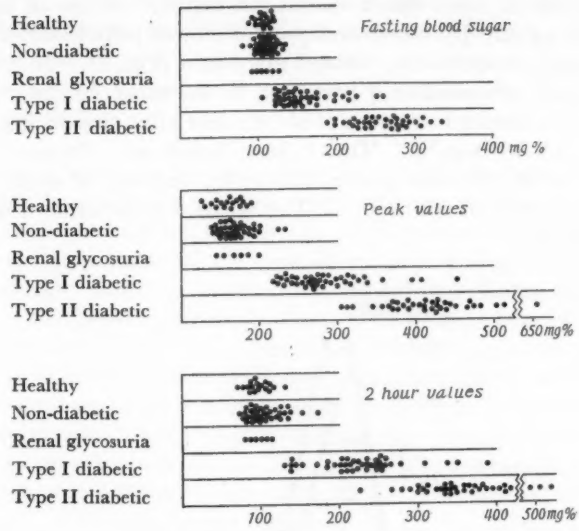

Fig. 1. Distribution of fasting blood sugar levels, peak levels and 2 hour levels in standard sugar loading test.

limit of 2 hour level was 120 mg.%, if M+2 S.D. was used. One of the 17 healthy adults showed a 2 hour blood sugar level of 134 mg.%, a peak level of 191 mg.% and a 4 hour level of 88 mg.%.

Our criteria for a normal sugar loading curve are: a fasting blood sugar below 120 mg.%, a peak level below 200 mg.%, a 2 hour level below 120 mg.% and a negative urine sugar. The only case of the 17 normal individuals who could not satisfy these criteria completely had a 2 hour level of 134 mg.%.

Evaluation of the sugar loading curve of non-diabetic patients groups

This group consisted of 37 various hospitalized patients who were thought to have impaired ability to assimilate carbohydrate but negative urine sugar. Their maladies were pancreatic diseases (cancer and inflammation), hepatic diseases (heaptitis, cancer of common bile duct), endocrine diseases (Addison's disease, thyrotoxicosis, myxedema, dwarfism, Simmonds' disease), and other diseases (gastric cancer, narcolepsia, muscular dystrophy, pulmonary tuberculosis). The mean fasting blood sugar was 100 mg.% (90–135 mg.%); and in contrast to the normal group, 7 of the 36 cases have a fasting level above 120 mg.% but included in 134 mg.% (M+2 S.D.) except one case, whose fasting value exceeded this level by 1 mg.%. The mean peak level was 165 mg.% (139–232 mg.%);

and 35 of the 37 cases didn't exceed 200 mg.%. A case of diabetes insipidus and a gastro-jejunostomized patient showed peak levels of 227 mg.% and 232 mg.% respectively, without glycosuria (Fig. 2). In 18 of the 37 cases the peak occurred at ½ hour and in the other 19 cases at 1 hour. The mean maximum increment of blood sugar after glucose ingestion, was 55 mg.% (21–101 mg.%). The 2 hour value was 106 mg.% (76–170 mg.%), 9 of the 37 cases above 120 mg.%, and one of them 170 mg.% with a peak level of 174 mg.%. The mean 4 hour value was 90 mg.% (63–144 mg.%).

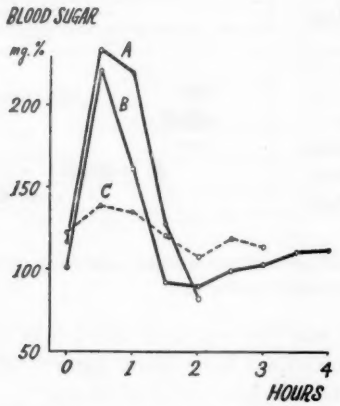

Fig. 2. Glucose loading curves. A . . . 48 year-old male, gastro-jejunostomized. B . . . 28 year-old male, diabetes insipidus. Both peak levels exceed 200 mg.%, but urine sugar excretions were negative. C . . . broken line represents the sugar loading curve of 20 year-old female, nephrosis. In this case four urine specimens including fasting one, gave positive sugar reaction.

Evaluation of the sugar loading curve in type I diabetes group

This group consisted of patients whose diabetic symptoms could be controlled by our high carbohydrate low fat diet alone (250–350 g. carbohydrate, 30–40 g. fat, 70–100 g. protein, 30–35 Cal/kg). The mean value of fasting blood sugar was 159 mg.% (104–256 mg.%) and that of 78% of type I diabetics ranged from 120 to 200 mg.%. 6 of 41 showed fasting levels above 200 mg.%; and one case showed a fasting level below 120 mg.%. The blood sugar rose rapidly to its highest level in 1 or 1½ hour and fell gradually to hypoglycemic levels. The hypoglycemia rose to the initial value very slowly. The mean peak value was 280 mg.% (218–452 mg.%). The maximum increment of blood sugar was 119 mg.%

(84–181 mg.%). The mean 2 hour value was 222 mg.% (142–378 mg.%), and there is no case whose 2 hour value was below 120 mg.%. The mean 4 hour value was 127 mg.% (69–227 mg.%). 10 cases showed a 4 hour value below 100 mg.% and 3 cases above 200 mg.%. In all cases urine sugar was positive.

Evaluation of the sugar loading curve in type II diabetes group

This group consisted of patients whose diabetic symptoms were controlled by insulin in addition of our high carbohydrate low fat diet. The mean fasting blood sugar of the type II diabetic group was 268 mg.% (187–472 mg.%); and 91% of this group showed their fasting value above 200 mg.%. The blood sugar rose rapidly to its peak at 1 or 1½ hour and decreased gradually. The hypoglycemia began to occur usually at 3½ hours. In 14 of the 35 cases hyperglycemia was protracted; and the blood sugar did not return to the fasting level within 4 hours. The mean value of a peak level was 415 mg.% (308–658 mg.%). In 21 of the 35 cases (60%) the peak value was above 400 mg.%. The maximum increment of blood sugar was 144 mg.% (94–220 mg.%). The mean 2 hour value was 366 mg.% (226–520 mg.%). In 30 of the 35 the 2 hour value was above 300 mg.%. The mean 4 hour value was 266 mg.% (160–406 mg.%); and in 31 of the 35 cases this was above 200 mg.%. In all cases, urine sugar was positive.

Evaluation of the sugar loading curve in renal glycosuria

Joslin applied this name to the condition in which the renal threshold is extremely low, as indicated by the fact that all specimens of urine examined, including those after an overnight fast, contain sugar.[6] Such cases are very rare. On the contrary, Hatlehol defined renal glycosuria as the presence of a positive urine sugar reaction without diabetic symptoms and the subsequent absence of true diabetes during prolonged observation.[7] In his paper there are some cases of renal glycosuria which have a hyperglycemic peak above 200 mg.%, in some cases 300 mg.%. We do not accept this definition. We define renal glycosuria as that condition in which the alimentary hyperglycemic peak and a 2 hour value are within normal limits but the urine sugar is positive.

The mean fasting blood sugar of our 6 cases of renal glycosuria was 107 mg.% (94–121 mg.%) and the mean peak level was 170 mg.% (139–198 mg.%). The 2 hour values ranged between 80 and 110 mg.%. In all cases urine sugar became positive after sugar ingestion, but the fasting urine specimens gave negative sugar reactions except in one case, whose fasting blood sugar was 121 mg.% and peak level was 139 mg.% (Fig. 2).

The differentiation of mild diabetes mellitus and renal glycosuria is

relatively easy if our definition of renal glycosuria is used. But this differentiation may be difficult, if an extremely low renal threshold for sugar (for example below 100 mg.%) is necessary for the diagnosis of renal glycosuira. If the latter definition is accepted, how shall we diagnose the case in which the sugar loading curve is normal and glycosuria appears only when the blood sugar is above 150 mg.%? Is this a mild case of diabetes or is this a so-called potential, latent or pre-diabetes? If such a case represents latent diabetes, the question arises whether lowered renal threshold or prolonged alimentary hyperglycemia is the essential disturbance in diabetes mellitus. It is hard to believe that the lowered renal threshold is a prodromal finding of diabetes mellitus. We feel that the slight increase in hyperglycemic peak exceeding the normal limit and/or the protraction of alimentary hyperglycemia may be an essential finding even in the latent stage of diabetes mellitus. Therefore, it is more proper to regard the patient who has glycosuria and a normal sugar loading curve as a renal glycosuric patient in whom there is normal sugar assimilation but an impaired reabsorption of sugar in the kidney. According to our opinion, the glycosuric patient, whose loading curve has a peak value below 200 mg.% and a 2 hour value below 120 mg.%, is a renal glycosuric patient in a broad sense; and the glycosuric patient, whose peak value in sugar loading curve is above 200 mg.% for capillary blood, is a mild case of diabetes mellitus, even if his glycosuria will disappear by a slight diet restriction. " Latent diabetes " or " potential diabetes " seems to be an unnecessary nomenclature. If these words are retained, we prefer to use the term " latent diabetes " for the patient, whose sugar loading curve has a peak value above 200 mg.% and a 2 hour value above 120 mg.% with negative urine sugars.

There are several theoretical explanations of renal glycosuria: the theory of decreased phosphorylation in tubuli contorti due to hypofunction of adrenal cortex; the pituitary hormone theory; the lability of the vegetative nervous system theory. Renal glycosuria was provoked clinically during ACTH treatment.[8] Yet the etiology is not clear.

On of our 6 cases, a 20 year-old woman, suffered from severe nephrosis. Her urine protein concentration was 30‰ and serum protein was 3.8%. Urine sugar was positive throughout her clinical course. Her sugar loading curve was normal although the fasting blood sugar was 121 mg.%, as shown in Fig. 2. It seems proper to call such a case secondary renal glycosuria, in contrast to idiopathic renal glycosuria in which other symptoms are not manifest.

Is it always possibile to differentiate diabetic and non-diabetic by the sugar loading test?

It is easy to distinguish the typical normal sugar loading curve from the diabetic curve, but we are sometimes confused in the diagnostic evaluation of an abnormal sugar loading curve, which does not satisfy the criteria of a normal sugar loading curve nor that of a diabetic curve. Some of these abnormal curves may originate in careless test conditions; such as previous starvation, carbohydrate restriction, diarrhea, insomnia, etc.

The transitional cases are those with an abnormal fasting blood sugar, a normal peak, and a normal 2 hour value; those with an abnormal peak level, a normal fasting, and a normal 2 hour value; and those with an abnormal fasting, an abnormal 2 hour value, but a normal peak level, etc. How shall we interprete such cases?

A high fasting blood sugar is observed in a healthy test subject, when he is tired or drowsy. A 29 year-old physician was examined on the morning after night duty and his fasting blood sugar found to be 147 mg.% for capillary blood and 146 mg.% for venous blood without glycosuria. Few weeks later when blood sugar examinations were made again two off-duty mornings his fasting blood sugar values were 106 mg.% and 94 mg.%. The fasting blood sugar level of a 22 year-old laboratory technician was determined every 10 minutes from 4:50 a.m. to 8:00 a.m. after night duty. The results are shown in Fig. 3. The blood sugars fluctuate from

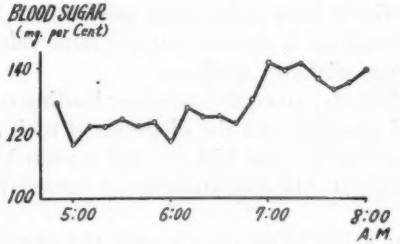

Fig. 3. Oscillation in the fasting blood sugar. 20 year-old male. 5:20 a.m. to 6:40 a.m., sleeping during part of this time.

116 to 144 mg.% within 3 hours. Later a sugar loading test confirmed his normal carbohydrate assimilating power. It was observed by Hatlehol[9] and Kuzuya[10] that the fasting blood sugar rises in the early morning. Therefore, if the blood sugar estimation is made before 8 a.m. a moderately high fasting blood sugar level does not always mean a diabetic condition. But if repeated estimations of fasting blood sugar show constant high values, we should regard such a case as diabetic.

When the hyperglycemic peak of the sugar loading curve exceeds 200 mg.%, should we regard the loading curve as diabetic? The normal hyperglycemic peak usually does not exceed 200 mg.%, but it is frequently

observed that some people show a hyperglycemic peak above 200 mg.% without glycosuria. It has been observed that old people have such curves, but they have no diabetic symptoms and do not develop true diabetes. It has been shown that the rapid absorption of sugar from the intestine causes a steep high-peaked hyperglycemic curve. Such a curve has been called a lag storage curve or oxyhyperglycemia.[5] We obtained such a curve in a case of diabetes insipidus and in a gastro-jejunostomized patient (Fig. 3). Their urine specimens gave a negative sugar reaction. Therefore, a hyperglycemic peak of over 200 mg.% does not always mean diabetes mellitus. However, non-diabetics cannot have a hyperglycemic peak above 230 mg.% in his loading curve, or at least above 250 mg.%.

Protraction of alimentary hyperglycemia is one of the characteristic findings in diabetes mellitus. In 12 of the 17 normal cases, and in 25 of the 36 non-diabetic patients, the hyperglycemia fell to fasting blood sugar levels within 2 hours; on the contrary, this occurred in only one case of the 41 type I diabetics and in none of the 35 type II diabetics. All of the diabetic cases in our series showed a 2 hour value above 140 mg.%, and 8 of the 36 non-diabetic patients showed a 2 hour value above 120 mg.%. These 8 cases consisted of 3 cases of strumitis, 2 cases of pancreatic cancer, one case each of lung cancer, cholelithiasis, and myelocytic leukemia. Only one case of the 17 normal adults showed a 2 hour value above 120 mg.%. Hence, if the 2 hour value does not exceed 120 mg.%, the diagnosis of diabetes mellitus is denied, but a 2 hour value above 120 mg.% does not always mean diabetes mellitus.

Speaking conclusively, an equivocal sugar loading curve is infrequently obtained in clinical practice, and the diagnosis of such a curve is difficult. Further clinical observation, past history, and repeated sugar loading test, are necessary to designate the case diabetic or non-diabetic.

Can we decide by means of the sugar loading test whether the diabetic patient will need insulin?

If we can differentiate type I diabetes and type II diabetes by means of sugar loading test at the beginning of hospitalization or in the ambulatory clinic, the need for insulin treatment in a given patient may be estimated in advance. For this purpose the present attempt was made to compare the blood sugar curves of these two diabetic groups.

If the criterion is made that the fasting value of type I diabetics is below 195 mg.% (M+S.D.) and that of type II diabetics above 218 mg.% (M−S.D.) 18.4% of our diabetic patients are misjudged, that is, 8 cases of the 41 type I diabetics and 5 of the 35 type II diabetics deviate from these two limit values. If the upper limit of 2 hour value for type I diabetics is 280 mg.% (M+S.D.), 5 of the 41 cases exceed this value; and

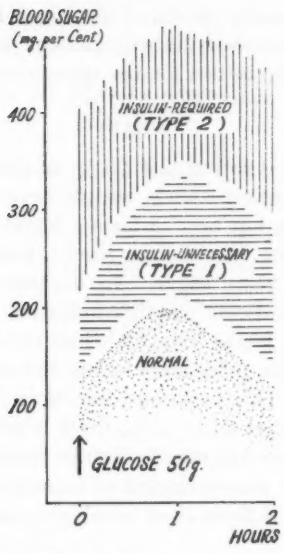

Fig. 4. Gross distribution of blood sugar values in sugar loading test among insulin-required type diabetes (type II), insulin-unnecessary type diabetes (type I), and normal group.

if the lower limit of that for type II diabetics is 298 mg.% (M—S.D.), 5 of the 35 cases are below this value. The upper limit of peak value for type I diabetics is 332 mg.% when M+S.D. is used and 6 of 41 cases exceed this level. The lower limit of peak value for type II diabetics is 348 mg.% when M—S.D. is used and 4 of 35 are below this value. If the criterion is made that the maximum urine sugar concentration of type I diabetics is less than 3.7% (M+S.D.) and the maximum sugar concentration of urine specimens for type II diabetics is more than 3.8% (M—S. D.), 8 of the 41 type I diabetics and 2 of the 35 type II diabetics deviate from these limit values.

If the criteria are made that in type I diabetics the fasting level is below 195 mg.%, the peak level below 332 mg.%, the 2 hour level below 280 mg.% and the maximum urine sugar concentration below 3.7%; and that in type II diabetics the fasting level is above 218 mg.%, the peak level above 348 mg.%, the 2 hour level above 298 mg.%, and the maximum urine sugar concentration above 3.8%, as shown in Fig. 4, 80% of our 76 diabetic patients are correctly classfied as type I or type II. Only one case of 41 type I diabetic patients satisfied the above criteria of type

II diabetes and 2 cases of the 35 type II diabetic patients satisfied those of type I diabetes. Thus by means of the sugar loading test we may determine whether the untreated diabetic patient will subsequently require insulin as well as dietary control for the management of his diabetes.

Summary

The standard sugar loading test (50 g. of glucose) was performed in normal adults, non-diabetic patients, and diabetic patients. Our critieria for normal sugar loading test are: a fasting blood sugar below 120 mg.%, a peak level below 200 mg.%, a 2 hour level below 120 mg.% for capillary blood, and a negative urine sugar. In diabetic patients, evaluation of the loading curve may indicate whether insulin is needed in the treatment schedule. If the fasting blood sugar is below 195 mg.%, the 2 hour value below 280 mg.%, and the peak level below 332 mg.%, his diabetic symptoms can be controlled without insulin injections; on the contrary, if the fasting blood sugar is above 218 mg.%, the 2 hour value above 298 mg.%, and the peak level above 348 mg.%, insulin must be used in addition to dietary treatment. The interpretation of transitional and equivocal sugar loading curves and the definition of renal glycosuria were also discussed.

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Limiting Level of Hyperglycemia in Diabetics

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Healthy adults have a fixed and unchangeable maximum level of hyperglycemia, which cannot be raised by oral administration of glucose.[1)2)] Recently, Saito and co-workers demonstrated that a fixed maximum level of hyperglycemia also exists in diabetic patients. They called this the "limiting level of hyperglycemia."[3)] In the present study this "limiting level of hyperglycemia" was determined in 46 diabetic and 5 non-diabetic patients.

Experimental

Method

Blood sugar levels were determined by the micromethod of Fujita and Iwatake.[4)] The limiting level of hyperglycemia was estimated from the results of one and two-dose glucose tolerance tests. In the one-dose test, 50 g. of glucose was given orally following an overnight fast. Blood specimens were collected from the ear lobe before and every 30 minutes for 3 hours after the administration of glucose. The two-dose test was performed within 3 days after the one-dose test. The procedure was the same, except that a second 50 g. dose of glucose was administered one hour after the first dose. Both the one-dose and two-dose glucose tolerance tests gave a "one-peaked" curve.

A "presumed" two-dose loading curve was constructed from the one-dose curve by adding the difference between the fasting level and values at $\frac{1}{2}$, 1, $1\frac{1}{2}$ and 2 hours, to the observed levels at $1\frac{1}{2}$, 2, $2\frac{1}{2}$ and 3 hours, respectively.

To obtain the limiting level of hyperglycemia, the actual two-dose loading curve is compared with the "presumed" two-dose curve. If the peak level in the actual test is less than the peak of the "presumed curve", or does not exceed the presumed curve by more than 10 mg.%, the peak level in the actual two-dose test is taken as the "limiting level of hyperglycemia" (Table I).

If the peak of the actual two-dose test exceeds the "presumed" peak level, it is likely that a three-dose or four-dose test would be needed to reveal the true "limiting level of hyperglycemia." Seven cases were encountered in whom the peak level of the actual test exceeded by more than 10 mg.% the "presumed"

TABLE I

Example of Calculation of "Presumed" 2-dose Glucose Loading Curve and "Limiting Level of Hyperglycemia"

	Blood sugar (mg. per cent)						
	Fasting	½	1	1½	2	2½	3
1-dose curve actual value	200	270	350	300	250	220	200
Actual value minus fasting blood sugar	–	70	150	100	50	20	–
Calculation of presumed 2-dose curve	–	–	–	300 +70	250 +150	220 +100	200 +50
Presumed 2-dose curve	200	270	350	370	400	320	250
Actual 2-dose curve	205	280	340	375	385*	300	260

* Limiting level of hyperglycemia.

peak. In these cases, the limiting level of hyperglycemia is indicated in Table II by a "$<$" sign ("greater than") preceeding the actual peak level.

In non-diabetic patients, the ability to assimilate glucose is so great that administration of glucose at one-hour interval does not indicate the limiting level of hyperglycemia. Therefore in the 5 non-diabetic patients the second dose of glucose was administered 30 minutes after the first dose.

In our clinic, diabetic patients are classified according to therapeutic requirements into two groups. Type I includes patients who are controlled by our high carbohydrate and low fat diet alone, and Type II includes patients who require insulin as well as dietary control.

Results

In all of the non-diabetics and in half (10 of 21) of the mild (Type I) diabetics the peak level in the two-dose procedure was approximately (within 20 mg.%) the same as the peak in the one-dose procedure. In the other words, the limiting level of hyperglycemia was reached in the one-dose test. This is in agreement with the observations of MacLean and deWesselow who found that increasing the single oral dose of glucose above 25 g. did not produce any further increase in peak blood sugar levels, although larger doses prolonged the hyperglycemic phase.

In half of the mild (Type I) and in 23 of 25 severe (Type II) diabetics the highest level in the two-dose procedure was more than 20 mg.% greater than the peak value in the one-dose test. In other words, the limiting level of hyperglycemia was not reached in the one-dose test. Furthermore, in 7 diabetic patients the measured peak in the two-dose test exceeded the expected additive effect of a second dose of glucose as calculated in the presumed two-dose curve. This phenomenon may be due to spontaneous decrease in endogenous glucose levels during the one-dose test,

so that the actual rise in blood sugar is greater than that calculated by subtracting the fasting blood sugar level from the observed values.

The relationship between the fasting blood sugar and the limiting level of hyperglycemia is depicted in Fig. 1. The relationship is linear; the higher fasting blood sugar levels are associated with proportionately higher values for the limiting level of hyperglycemia.

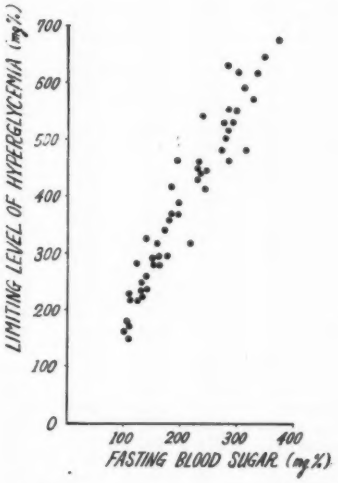


Fig. 1. The relationship between limiting level of hyperglycemia and fasting blood sugar.

The median value for the ratio of limiting level of hyperglycemia to fasting blood sugar fell between 1.8 and 1.9 and 90% of the ratios fell between the values of 1.6 and 2.3 and 35 of the 51 ratios fell within the narrow limits of 1.7 to 2.0.

As would be expected, the limiting level of hyperglycemia was lowest in non-diabetics and highest in Type II diabetics.

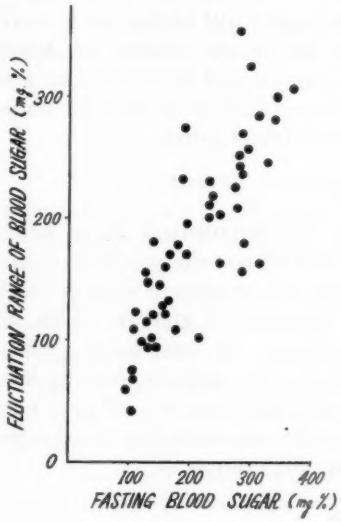
Here we would like to introduce the term " fluctuation range of blood sugar." This is meant to indicate the fluctuation in blood sugar occurring from a given stimulus, in this instance oral administration of glucose. By definition, it is the difference between the limiting level of hyperglycemia and the fasting blood sugar. (We realize this is only an approximation, as the hypoglycemic phase following oral ingestion of glucose usually will be less than the fasting blood sugar.)

The relationship of the fluctuation range of blood sugar to the fasting blood sugar level is shown in Fig. 2. There is a clearcut relationship;

TABLE II

Case No.	Age Sex	Disease	Blood Sugar (mg. per cent)						limit. level / fasting
			1-dose test			2-dose test		Limiting level of hyperglycemia	
			Fasting	Max. value	Presumed peak	Fasting	Measured peak		
1	52F	Diabetes mellitus (II)	472	658	676	368	673	673	1.82
2	69F	Diabetes mellitus (II)	368	452	518	326	570	<570	1.74
3	55F	Diabetes mellitus (II)	336	484	594	344	644	<644	1.87
4	62F	Diabetes mellitus (II)	336	470	554	314	476	476	1.51
5	64M	Diabetes mellitus (II)	334	466	552	274	526	526	1.91
6	61M	Diabetes mellitus (II)	326	504	648	308	590	590	1.91
7	70F	Diabetes mellitus (II)	308	528	680	334	614	614	1.83
8	55F	Diabetes mellitus (II)	300	480	598	294	618	<618	2.10
9	57F	Diabetes mellitus (II)	288	438	498	274	500	500	1.82
10	61F	Diabetes mellitus (II)	284	448	532	288	530	530	1.84
11	15M	Diabetes mellitus (II)	272	462	621	274	628	628	2.29
12	55M	Diabetes mellitus (II)	270	420	508	292	548	<548	1.87
13	62M	Diabetes mellitus (II)	266	372	483	272	480	480	1.76
14	44F	Diabetes mellitus (II)	266	388	504	280	514	514	1.83
15	64M	Diabetes mellitus (II)	264	358	428	228	428	428	1.87
16	51M	Diabetes mellitus (II)	254	342	394	288	445	<445	1.54
17	63F	Diabetes mellitus (II)	256	452	573	280	552	552	1.97
18	50M	Diabetes mellitus (II)	248	360	404	191	388	388	2.03
19	43F	Diabetes mellitus (II)	246	382	478	229	440	440	1.92
20	35F	Diabetes mellitus (II)	240	440	566	233	541	541	2.32
21	44M	Diabetes mellitus (II)	233	292	328	188	462	<462	2.40
22	51F	Diabetes mellitus (II)	230	368	501	242	444	444	1.83
23	54M	Diabetes mellitus (II)	228	378	452	246	410	410	1.66
24	43M	Diabetes mellitus (I)	224	312	386	215	316	316	1.46
25	56F	Diabetes mellitus (II)	214	372	452	284	462	462	1.62
26	55M	Diabetes mellitus (II)	214	396	528	228	458	458	2.00
27	57M	Diabetes mellitus (I)	214	298	362	179	358	358	2.00
28	54M	Diabetes mellitus (I)	193	278	343	192	364	<364	1.89
29	58F	Diabetes mellitus (I)	174	259	307	175	292	292	1.66
30	47M	Diabetes mellitus (I)	174	266	348	139	322	322	2.31
31	50M	Diabetes mellitus (I)	167	337	467	166	334	334	2.03
32	60M	Diabetes mellitus (I)	168	254	312	147	292	292	1.98
33	56M	Diabetes mellitus (I)	160	274	388	125	268	274	2.19
34	62M	Diabetes mellitus (I)	160	240	292	153	280	280	1.83
35	70F	Diabetes mellitus (I)	157	308	404	155	318	318	2.05
36	50M	Diabetes mellitus (I)	154	245	354	160	291	291	1.81
37	53M	Diabetes mellitus (I)	154	257	356	135	242	257	1.90
38	53M	Diabetes mellitus (I)	154	327	481	180	414	414	2.30
39	53F	Diabetes mellitus (I)	146	233	309	158	280	280	1.76
40	57F	Diabetes mellitus (I)	138	224	270	130	212	224	1.72
41	43F	Diabetes mellitus (I)	128	227	280	135	235	235	1.74
42	56F	Diabetes mellitus (I)	121	266	368	121	278	278	2.29
43	58M	Diabetes mellitus (I)	121	247	380	128	238	247	1.92
44	54M	Diabetes mellitus (I)	104	215	268	107	217	217	2.02
45	53M	Diabetes mellitus (I)	104	189	218	132	228	228	1.72
46	49M	Diabetes mellitus (I)	99	200	286	121	219	219	1.80
47	43M	Renal glycosuria	106	160	177	99	161	161	1.62
48	37M	Renal glycosuria	94	174	224	102	177	177	1.73
49	28M	Muscular dystrophy	115	174	206	104	170	174	1.67
50	27M	None	112	145	178	106	148	148	1.39
51	33F	Anorexia nervosa with hypo-insulinism	100	226	291	102	214	226	2.21

the higher the fasting blood sugar, the greater the fluctuation range of blood sugar. This relationship is not seen in the usual one-dose glucose tolerance test; the maximum blood sugar increase in the one-dose test does not increase linearly in proportion to the fasting blood sugar level (Fig. 3). The fluctuation range of blood sugar of Type II diabetics usually exceed 200 mg.%. In Type I diabetics the value usually falls between 100 and 200 mg.% and in non-diabetics the fluctuation range is less than 100 mg.%.



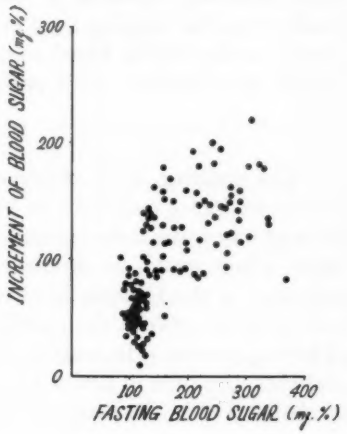


Fig. 2. The relationship between fluctuation range of blood sugar and fasting blood sugar.

Fig. 3. The relationship between the maximum increase in blood sugar after oral administration of 50 g. glucose and fasting blood sugar. This result was obtained in our previous investigation.[7]

Discussion

The results of this study demonstrate that a " limiting level of hyperglycemia " exists in the diabetic patients just as in the normal individual. Moreover, the limiting level of hyperglycemia bears a close relationship to the fasting blood sugar level in both diabetic and non-diabetic subjects. The mechanism of this limiting level of hyperglycemia is not apparent, but we feel the limiting level represents the summation of the assimilating functions, including intestinal absorption, the many enzymatic functions of the liver, the endocrine and, indirectly, exocrine functions of the pancreas,

and the excretory and enzymatic functions of the kidney.

It should be emphasized that determination of the limiting level of hyperglycemia has no diagnostic or prognostic application in the usual case of diabetes mellitus, where the fasting blood sugar and one-dose glucose tolerance test will suffice for diagnostic purposes.

Our previous[5),6)] and present results serve to emphasize the importance of dietary regulation in the management of diabetes mellitus. Although, for a given fasting blood sugar value, there exists predictable limiting level of hyperglycemia regardless of oral intake of carbohydrate, the higher the fasting, or initial, blood sugar level, the higher will be the rise in blood sugar following ingestion of food. From the present studies we would predict that the limiting level of hyperglycemia would be reduced in proportion to the fasting blood sugar as the severe diabetic is brought under control with insulin. This problem is being investigated.

Summary

The limiting level of hyperglycemia was determined by a 2-dose glucose tolerance technique in 46 diabetic and 15 non-diabetic individuals. In most cases, the 2-dose technique revealed the maximum level of blood sugar which could be attained by oral ingestion of glucose. This, by difinition, is the limiting level of hyperglycemia. A remarkably constant ratio existed between the fasting blood sugar value and the limiting level of hyperglycemia. In both severe and in mild diabetics, as well as in non-diabetics, the ratio of limiting level of hyperglycemia to fasting blood sugar levels fell within the range of 1.6 to 2.3 in 90% of cases.

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The Influence of Radioactive Dust on Experimental Pneumoconiosis

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Introduction

The inhalation of quartz dust may be the prime cause in the pathogenesis of silicosis, but it is also believed that the susceptibility of lung tissue to the impingement of quartz dust is of some importance for the development of the disease.

For this reason, the author has studied the other influencing factors than the dust inhalation itself, and had an opportunity to find a marked development of fibrotic nodules in the lungs of experimental animals made to inhale the quartz dust and X-ray irradiated.

The results of this experiments have been pulished in the Tohoku Journal of Experimental Medicine.[1)]

This work called my attention to the hypothesis of Dantin and Gallego[2)] in 1950 that the natural radioactivity of the rocks may play a part in the aetiology and pathogenesis of silicosis, and led me to an experimental research of the hypothesis, for there is no experimental work on this problem.

However, it seems to me an important thing to see whether the radioactive dust plays a part to the pathogenesis of the silicosis or not, not only as a researcher on silicosis, but also as a suffering human being under menace of terrible aero-dust produced by the explosion of atomic bombs of the atomic age.

Experimental

Method

The author intended to know the influence of radioactivity through experimental pneumoconiosis, and it has been thought better to use some of the natural radioactive silicates comparing with non-radioactive silicates.

The experimental procedures were divided into two parts. The first series

of experiments was to see the effect of the low natural radioactivity on pneumoconiosis and the second was to see that of the higher natural radioactivity. Low radioactive feldspar was used for the first purpose, and high radioactive allanite for the second.

Beside the principal examination through inhalation of these dust, each experiment was preceded by preliminary examination by way of the subcutaneous and intraperitoneal injection of suspension of these dusts.

Description of dust materials

The low radioactive feldspar was obtained from Ishikawa uranium mine field in Japan. The control feldspar, non-radioactive and almost similar in its chemical composition was obtained from Bunkei mine field in Korea.

The result of chemical analysis of radioactive and non-radioactive feldspars was shown in Table I.

TABLE I

Chemical Analysis of Radioactive Feldspar and Control Feldspar

	Radioactive feldspar (Ishikawa mine field)	Non-radioactive feldspar (Bunkei mine field)
Ignition loss	0.65	0.62
Tolal SiO_2	67.90	68.54
Al_2O_3	17.90	17.50
Fe_2O_3	0.06	0.13
CaO	0.65	0.80
MgO	trace	trace
Na_2O	3.32	4.27
K_2O	9.49	8.02
*Free SiO_2	0.04	0.08

* Free silica is analysed by means of N.A. Talvite's method.

The high radioactive allanite for the second series of experiments was produced in Ishikawa uranium mine field. The control allanite was also obtained from near the Ishikawa uranium mine field. The result of chemical analysis of the high radioactive allanite and its control allanite was as shown in Table II. Radioactivity of samples were presented in Table III and IV.

Besides, alpha and beter tracks were seen in the autoradiograms of the radioactive feldspar and allanite.

Experimental animals

For study with feldspar, sixty-eight rats, either male or female of average weight of 180 gms were used. They were divided into two groups, one group of fourty-six rats for inhalation and the other group of twenty-four rats for subcutaneous and intraperitoneal injection.

For study of allanite, sixty rats, either male or female of average weight of 180 gms were used. They were also divided into two groups. The first of thirty-six rats were used for inhalation, the other twenty-four rats were used

TABLE II

Chemical Analysis of Radioactive Allanite and Its Control Allanite

(Mostly quoted from the analytical figures by K. Kimura and E. Minami)

	Radioactive allanite (Ishikawa uranium mine)	Control allanite (in near Ishikawa field)
Total SiO_2	32.97	30.40
Al_2O_3	14.51	15.45
Fe_2O_3	—	3.58
Ce_2O_3	4.87	8.87
ThO_3	1.64	0.24
VO_2	trace	—
FeO	15.23	11.47
MnO	4.32	2.63
MgO	0.74	0.06
CaO	12.61	0.82
H_2O	4.58	2.05
SnO_2	trace	0.66
CO_2	0.11	0.33
Free SiO_2	0.05	0.06

TABLE III

Radioactivity of Samples of Radioactive Feldspar and Its Control Feldspar

	C.P.M. (counts per minute) for 28 mm. Distance
Radioactive feldspar (1 g)	78.0
Control feldspar (1 g)	24.0
Mean back ground	21.0

TABLE IV

Radiocctivity of Samples of Radioactive Allanite and its Control Allanite

	C.P.M. for 28 mm. distance
Radioactive allanite (1 g)	822.0
Control allanite (1 g)	54.0
Mean back ground	21.0

for the test of subcutaneous and intraperitoneous injection.

Procedure of inhalation and injection of dusts

The silicates, feldspar and allanite, were pulverized in a ball mill until

the dusts had 80% of the particles below 3 microns in diameter. These dusts were dried in a sterillizer for half an hour at 120°C immediately before use. The animals were made to inhale in a dust cabinet under high dust concentration. The dust in the cabinet was checked from time to time during the inhalation with a dustcounter and was held at values in the range of 11,000 to 6,000 particles per c.c.

In the case of injection, 2 gms of particles below 2 microns equivalent diameter each of the silicate dusts were shaken in a bottle containing 20 ml of isotonic saline and were sterilized by autoclaving for 60 minutes at 15 lb. pressure. One c.c. each of the dust suspension, corresponding to 100 mgs. of dust was injected.

Pathological technique

Routine postmortem examinations were carried out whenever an animal died or was sacrified. The lungs of dead or killed animal were gently distended by injecting about 10 ml of 10% formolsaline through the trachea which was exposed at the neck. Most of the air in the lungs was driven out by a gentle pressure on the sides of the thorax. The trachea was ligatured, the thoracic cavity was opened and the lungs, with the tied-off portion of the trachea, were removed and placed in the fixation for four days, blocks were selected in the usual manner, cut into sections and stained with haematoxylin-eosin and malloryazan or Bielschowsky stains.

Results

The pathological changes produced in the lungs of the animals have been assessed according to Belt and King's[8] classification. Five grades of fibrosis have been recognized as follows: Grade 1, loose reticulin fibers with non ocollagen formation; Grade 2, compact reticulin fibers with or without collagen formation; Grade 3, slightly cellular, but almost collagenous; Grade 4, wholly collagenous and completely acellular, and Grade 5, acellular, collagenous, and confluent. The details of the individual experiments have been summarized in Table V, VI, VII, VIII and IX.

I. Experiments with feldspar dust

The pathological changes following subcutaneous and intraperitoneal injections of feldspar dust.

In the group receiving the radioactive feldspar subcutis, a severe degree of fibrosis (Frade 4) appeared in the subcutanous tissue as early as within two months. This nodules were 6 mm in diameter in size. Histologically, there was a marked necrosis in the centre of these nodules.

By 120 days, the fibrosis of these nodules was more advanced than that of the quartz fibrosis. In the case of intraperitoneal injection, a multiplicity of the nodules was produced on the peritoneum. The nodules were of a size of about 1 mm in diameter. These nodules were formed within two months. There were some large nodules of 3 mm in diameter among these nodules. The degree of fibrosis of these nodules was mostly Grade 3. and so severe as in the case of subcutaneous injection.

TABLE V

Assessment of Fibrosis Following Subcutaneous Injection of Radioactive Feldspar and Non-radioactive Feldspar

			Radioactive feldspar		Non-radioactive feldspar	
Days of survival	Rat No.	Mode of death	Size of nodule (mm.)	Grade of fibrosis	Size of nodule (mm.)	Grade of fibrosis
1- 30	1	K	5	3	0	0
1- 30	2	K	6	4	2	1
31- 60	3	K	6	3	2	3
31- 60	4	K	7	4	3	3
61- 90	6	K	6	4	2	3
61- 90	8	K	7	4	3	3
91-120	9	K	7	4	4	3
91-120	10	K	8	4	5	3
121-150	11	K	6	4	3	3
121-150	12	K	7	4	4	3
151-180	14	K	8	4	4	4
151-180	15	K	8	5	6	3

K ; killed

TABLE VI

Assessment of Fibrosis Following Intraperitoneal Injection of Radioactive Feldspar and Non-radioactive Feldspar

	Radioactive feldspar				Non-radioactive feldspar			
Days of suruival	Rat No	Made of death	Size of nodule (mm.)	Grade of fibrosis	Rat No	Mode of death	Size of nodule (mm.)	Grade of fibrosis
1- 30	17	K	1	2	19	K	0	0
1- 30	18	K	2	3	20	K	2	1
31- 60	16	K	2	3	21	K	1	2
31- 60	5	K	3	3	22	K	2	2
61- 90	23	K	2	3	24	K	1	2
91-120	7	K	2	4	25	K	2	3
61-120	13	K	2	4	26	K	2	3
91-120	10	K	2	3	27	K	2	3
191-150	28	K	2	4	31	K	2	3
121-150	30	D	2	4	34	E	-	-
151-180	32	K	2	4	35	D	2	4
151-180	33	K	2	4	36	E	-	-

K: killed. D: died. E: eaten.

Table VII

Pulmonary Alteration of Rats Exposed to Radioactive Feldspar Inhalation

Days of survival	Rat No.	Days of inhalation	Mode of death	Grade of fibrosif
99	106	21	D	1
99	107	21	K	1
100	105	21	E	1
100	108	21	E	–
100	110	21	D	1
100	111	21	D	1
130	114	32	K	1
130	115	32	K	1
226	116	111	K	2
237	103	96	D	2
243	102	70	D	3
243	118	116	K	3
330	120	140	K	2
330	121	140	K	2
367	122	140	K	3
367	123	140	K	3
386	101	143	K	3
386	124	143	K	3
441	125	148	D	3
491	130	186	K	3
493	134	186	K	3
493	126	186	K	2
546	128	200	K	3
550	133	200	E	–
550	131	200	K	3
550	129	200	K	3
560	104	64	K	3
560	117	64	K	3
560	119	64	K	3
564	132	200	K	4
564	127	200	K	3

K: killed. D: died. E: eaten

Table VIII

Pulmonary Alteration of Rats Exposed to Non-radioactive Feldspar Inhalation

Days of survival	Rat No.	Days of inhalation	Mode of death	Grade of fibrosis
42	201	27	K	0
42	202	77	K	0
128	203	77	K	0
129	204	77	K	1
187	205	77	K	0
285	208	141	K	1
330	209	141	K	1
384	210	141	K	1
463	211	162	K	1
528	212	218	K	2
530	213	218	K	2
541	206	79	K	2
541	207	79	D	2

K: killed. D: died

TABLE IX

Pulmonary Alteration of Rats Exposed to Radioactive Allanite Inhalation and Its Control Allanite Inhalation

Experimental group					Control group		
Days of survival	Rat No.	Days of inhalation	Mode of death	Grade of fibrosis	Rat No.	Mode of death	Grade of fibrosis
1- 30	301	17	K	1	311	K	0
31- 60	302	33	K	1	312	K	0
61- 90	303	50	K	2	313	K	0
91-120	304	63	K	2	314	K	0
121-150	305	70	K	2	315	K	0
151-180	306	88	K	2	316	K	0
181-210	307	126	K	3	317	K	1

K: killed

In the control group, receiving non-radioactive feldspar, there were no nodules after subcutaneous or intraperitoneal injection in two months. In three months, the first outset of fibrotic nodules appeared subcutis, in which the largest one measured as large as 4 mm in diameter.

The fibrotic progress of these nodules was not so rapid as in the case injected with radioactive feldspar, and the fibrosis reached Grade 3. In four months and six months, the degree of these fibrotic nodules reached Grade 4. The peritoneal processes were somewhat weaker in effect than the subcutaneous. The details of the individual experiments have been summarized in Table V and VI.

Pulmonary alteration of rats exposed to feldspar inhalation

Macroscopic appearances (experimental group receiving radioactive feldspar dust): The naked-eye appearance of lungs and pleura in this group made to inhale radioactive feldspar was quite normal and similar to that of the control group within six months. From nine months on, graysh-white spots of about 2 mm in diameter were seen on the surface of the lungs, mainly on the posterior sides. After 550 days the nodules increased in size and became more and more firm, and large patches of confluent fibrosis were seen involving the major part of the lung parenchyma.

Control group (receiving non-radioactive feldspar dust): There were no recognisible changes on the naked-eye appearance of lungs and pleura in this group even in one year and more.

Microscopic appearances (experimental group receiving radioactive feldspar dust): In these lungs, small granuloms were to be seen as early as in the fourth month, and dust cells were aggregated around the small arteries and the small bronchi. At this stadium, some loose reticulin fibers were already seen in the maculae of dust cells. On continuing the experiment, the number and size of these aggregates of dust cells increased, and the loose reticulin fibers became more and more numerous and compact. In eight months, fibrotic

Fig. 1

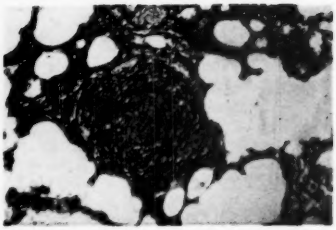
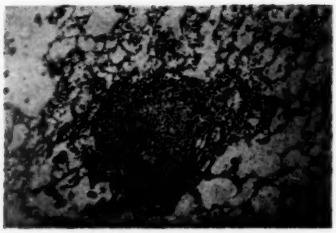


Fig. 2



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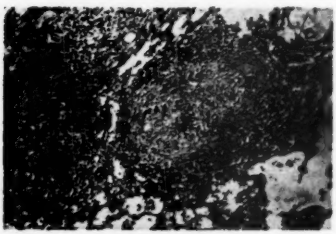

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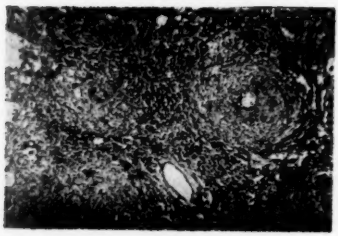


Fig. 1. Lung section from a rat inhaled radioactive feldspar showing compact reticulin fibers in the nodule (Grade 2.). Silver impregnation, ×100. Inhalation of radioactive feldspar dust (days of survival : 243 days ; days of inhalation : 70 days).

Fig. 2. Fibrotic nodule made up of dense collagenous fibers. The periphery of the nodule shows loose reticulin fibers (Grade 2.). Hx. E. ×70. Inhalation of radioactive feldspar dust (Days of survival : 330 days ; days of inhalation : 140 days).

Fig. 3. Lung section showing compact reticulin fibers and some collagenous fibers in the nodules (Grade 3). Hx. E. ×70. Inhalation of radioactive feldspar dust (Days of survival : 493 days ; days of inhalation : 186 days).

Fig. 4. Lung section showing fully collagenous and confluent fibrotic nodules (Grade 4). Hx. E. ×70. Inhalation of radioactive feldspar dust (Days of survival : 564 days ; days of inhalation : 200 days).

nodules with compact reticulin fibers were produced in a part of the lung tissue, mainly in the subpleura, and the interstitium became thickened. During 400 to 500 days the compact reticulin fibers were replaced by a collagen, but these changes were only partial in the lung tissue.

Even after 560 days, abundant dust cells were still seen and the hyaline parts or collagen element were still rather scanty.

Control group receiving non-radioactive feldspar dust : The histological changes in the lungs were first seen only in 400 days and only slightly. The recognisable pathological changes were only the aggregation of dust cells in the alveolar wall and around small arteries or small bronchi.

Fig. 5

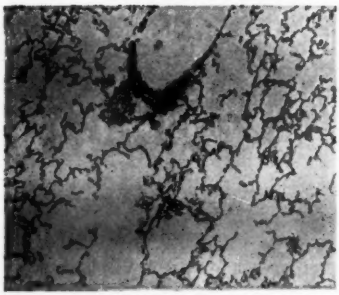


Fig. 6

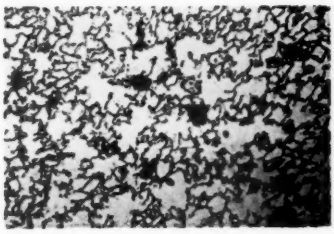


Fig. 7

Fig. 8

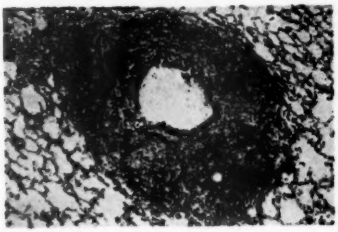


Fig. 5. Lung section showing only slight aggregation of dust cells in lung tissue (Grade O.). Hx. E. ×70. Inhalation of control non-radioactive feldspar dust (Days of survival : 285 days ; days of inhalation : 141 days).

Fig. 6. Lung section showing evident many granuloma with loose reticulin fibers and compact reticulin fibers (Grade 1.). Hx. E. ×70. Inhalation of control feldspar (Days of survivial : 530 days ; days of inhalation).

Fig. 7. Lung section of a rat inhaled radioactive allanite dust showing severe fibrotic lesion on the periphery area of the bronchioles. The grade of these fibrosis on this nodule extend to Grade 2, fibrosis. Hx. E. ×40. Inhalation of radioactive allanite dust (Days of survival : 66 days ; days of inhalation : 50 days).

Fig. 8. Lung section of a rat showing a round nodule with reticulin fibers on the periphery area of the bronchioles (Grade 2). Silver impregnation ×70. Inhalation of allanite dust (Days of survival : 72 days ; days of inhalation ; 50 days).

II. Experiments with allanite dust

The pathological changes following subcutaneous and intraperitoneal injection of allanite dust

In the experimental group receiving radioactive allanite dust into subcutis and the peritoneum cavity, a severe lesion occurred as early as in one month. The nodules after subcutaneous introduction were produced at an extremely rapid rate. The notable change of these nodules was severe necrosis in the centre of them. In 60 days, the centre of these nodules became soft and formed caverns.

Fig. 9

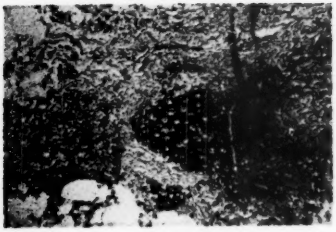


Fig. 10

Fig. 11

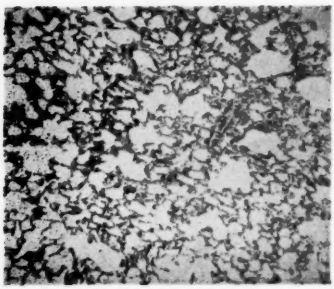


Fig. 12

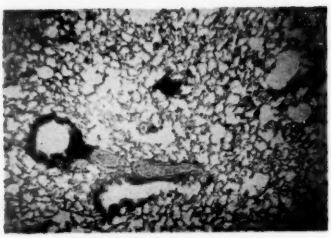


Fig. 9. Lung section of a rat showing a remarkable fibrotic lesion on the periphery of the nodule with accumlation of dust cells (Grade 2). Silver impregnation ×70. Inhalation of radioactive allanite dust (Days of survival ; 120 days ; days of inhalation : 70 days).

Fig. 10. Remarkable damage in the lung tissue of a rat inhaled radioactive dust (Grade 3). Hx. E. ×70. Inhalation of radioactive allanite dust (Days of survival : 208 days ; days of inhalation : 126 days).

Fig. 11. Photomicrograph of the rat lung tissue inhaled control dust not showing notable pathological changes without some accumulation of dust cells in alveolus. Hx. E. ×70. Inhalation of control allanite dust (Days of survival : 125 days ; days of inhalation. 70 days).

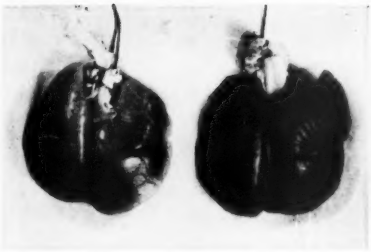
Fig. 12. Photomicrograph of the rat lung tissue showing some granuloma with weak reticulin fibers and dust cells (Grade 1). Hx. E. ×70. Inhalation of control allanite dust (Days of survival : 208 days ; days of inhalation : 126 days).

Dense compact reticulin fibers were seen on the outside of these caverns. The average size of these nodules of this stadium was 7 mm in diameter. In all, the degree of the lesion was more severe than that due to radioactive feldspar dust.

In the control group receiving slight radioactive dust, there were no notable lesions in subcutis and intraperitoneum cavity within two months.

By five and six months, many small nodules with about 2 mm in diameter were seen in the subcutis and intraperitoneum cavity. However, the necrosis in these nodules due to this control allanite dust was not so severe as the case of

Fig. 13



A B

Fig. 14

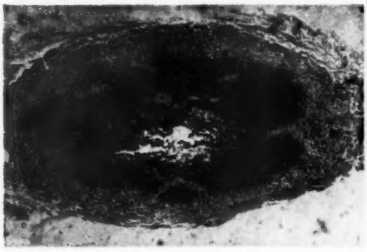


Fig. 13. A. Rat lung inhaled radioactive allanite dust (Days of survival: 50 days; days of inhalation: 33 days). A remarkably nodular spots are seen on the surfaces.

B. Rat lung inhaled control allanite dust (Days of survival: 50 days; days of inhalation: 33 days). No nodular spot on the surfaces.

Fig. 14. Showing the nodule after subcutaneous injection of 1 cc. of a suspension containing 100 mg. of radioactive allanite dust. Hx. E. ×20.

radioactive allanite dust.

Pulmonary alteration of rats exposed to allanite dust inhalation

Macroscopic appearances: In this experimental group receiving radioactive allanite, there appeared many nodular spots on the surface of the lungs in one month and in later months. These nodular spots increased steadily in size and became firmer until the final experiment.

In control group, no nodular spot was seen on the surface of the lungs even in six or seven months. There was no evidence of fibrosis throughout the lobes until the end of experiment.

Microscopic appearances: In the lungs receiving radioactive allanite dust, a conspicuous difference could be observed in the lung tissue between this experimental group and the control. A progressive severe pulmonary fibrosis was seen in the lung tissue of the former group.

After 50 days these nodules showed well-formed features and, what is more, lymph nodes were observed between the vessels and the bronchi. Compact reticulin fibers were seen in these lymphnodes in part. Progressive granulomata were almost always produced in the surroundings of the dilated vessels of the lungs. The dilation of vessels was seen extensively over the lungs. But the collagen formation of the nodules was not so much as to notice. In 71 days, these nodules became larger and multiple. The fibrosis in the lymphnodes advanced more and more, but collagen fibers did not develop so intensively as seen in the case that inhaled quartz dust. At 120 days after inhalation, the features of the nodules were confluent and the number of reticulin fibers inceased. In 188 days, they became fibrotic nodules of the grade 3 fibrosis in a part of the lungs. This degree of progress had a striking contrast with that of the control lungs.

In control group, dust particles lay freely with commencing aggregation in the alveoli by 90 days of experiment. In 180 days, the fibrotic feature was not seen throughout the lung tissue but aggregations of dust particles were found in the alveoli. In 180 days and after, these aggregates were somewhat compact and held together by fine reticulin fibers. The details of the individual experiments have been summarized in Table IX.

Discussion

It is generally known that many silicates except asbestos are inert to the lung tissue.

However, from the experimental results with feldspar dust, I have come to know that natural radioactive feldspar make fibrosis in animal lungs by artificial introduction of its dust. The control lung made to inhale nonradioactive feldspar did not show any fibrotic nodule.

From these results of the first experiment, it is supposed that the natural radioactivity may have an effect to cause fibrosis of the lungs.

To ascertain this, the second experiment was undertaken with allanite, more radioactive than the former. This second experiment induced a more remarkable fibrosis of the lungs in a relatively short time.

By these results, I could asertain that radioactive silicate had an agency to cause fibrosis in the lung tissue when the dust was inhaled into the lung.

These silicates, radioactive feldspar and allanite, had only a trace of free silica in the composition, so we can consider that these fibrosis was mainly caused by the natural radioactivity of these silicates.

In a former paper appearing in this journal, the author has demonstrated that X-ray irradiation could cause a rapid and intensive development of experimental silicosis. Therefore it seems not unreasonable that the natural radioactivity would influence the susceptibility of the lung to fibrosis in a similar way as X-ray. So that the Dantin and Gallego's theory was demonstrated by these results.

Conclusion

1. Experimental pneumoconiosis by inhalation of slight radioactive feldspar dust was developed in form of progressive fibrotic nodules in animal lungs.

2. Experimental pneumoconiosis by inhalation of control nonradioactive feldspar dust was negative to make fibrotic nodules in the corresponding periods.

3. Experimental pneumoconiosis by high radioactive allanite dust was rapidly and intensively developed in form of fibrotic nodules in animal lungs within short time.

4. Experimental pneumoconiosis by control allanite with a slight

radioactivity was not so severe, and the process of the fibrosis was slow and weak in comparison with the preceding case.

5. From these results mentioned, we might conclude that the natural radioactivity of rocks, when its dusts are inhaled, will cause the formation of fibrotic nodules in the lungs.

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Effect of Hypothermia on the Secretion of 17-Hydroxycorticosteroids of the Adrenal Glands in Non-Anesthetized Dogs

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The present investigation aims to know how hypothermia affects the secretion of 17-hydroxycorticosteroids of the adrenal glands in non-anesthetized dogs.

The experiments were performed in 5 dogs. The adrenal venous blood was collected by the lumbar route method of Satake, Sugawara and Watanabe[1], without anesthetizing or even fastening the animals. The plasma was separated from the collected blood samples by centrifugation and was analyzed for 17-hydroxycorticosteroids by the method of Nelson and Samuels[2]. In order to induce hypothermia, the animals were immersed into ice-water for 19–45 minutes and were then taken out. The body temperature fell markedly from 38.8–40.0°C., its lowest level (26.3–32.0°C.) being noted 5–25 minutes after the withdrawal from the ice-water bath. Then it rose gradually.

The basal rate of 17-hydroxycorticosteroids secretion was determined as 0.17–0.48 μg. per kg. of body weight per minute, the average being 0.30 μg. After the cold application it increased invariably, 0.35–1.0 μg. being reached. Compared with the initial rate in respective cases, the maximum was 1.7–2.9 fold. In 2 out of 5 cases an increase in the secretion rate was observed soon after the withdrawal and the maximum was reached within 20 minutes or so. In 3 others, however, an increase was preceded by a 20–60% decrease in the secretion rate, the maximum increase being observed within 1–2 hours.

These experimental results are not in consonance with those obtained by Egdahl, Nelson and Hume[3] in anesthetized dogs. In their experiments only definite fall in the 17-hydroxycorticosteroids secretion rate was observed.

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Studies of High-molecular Placental Components in Connection with Pregnancy Toxemia

Sixth Paper: Pentosenucleic Acid*

By

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The pentosenucleic acid was prepared starting with "Crude PNA I" and "II", which had been obtained and set aside when itinsulfuric acids were isolated from placentas.[1] The materials were fractioned at pH 6.8 with barium acetate and at pH 6.0 with calcium chloride to free from the contaminating DNA. The preparation from "Crude PNA I" (Prep. I) contained less than 5% DNA but that from "Crude PNA II" (Prep. II) even more than 30% DNA. However, Prep. I could be applied only for skin reaction because of shortage of material, and other biological assays were made on Prep. II. Judging from the results in conjunction with those on DNA[2], placental PNA injures not only liver but also kidney in pregnant rabbits like as Protein III (See a forthcoming paper[3]) in distinction from other high-molecular placental components including DNA. Kidney lesion is one of the changes in visceral organs characteristic of pregnancy toxemia.

Experimental

Preparation Procedure

"Crude PNA I" and "Crude PNA II" in the foregoing communication[1] were employed as materials. *Prep. I.* "Crude PNA I", which had been separated after extraction of the tissue brei with 0.14 *M* NaCl, was suspended in 30 cc. of water and dissolved by adding 1% NaOH to pH 6.8 under agitation, and 10 cc. of a 20% solution of crystalline barium acetate adjusted to pH 6.8 with acetic acid were added. The mixture was placed in an ice-box overnight, and the white flocculent precipitate was centrifuged off and washed with 40 cc. of 5% barium acetate (crystal-

* 11th information of "Nucleic Acids" by Masamune and co-workers.

line) of pH 6.8. Redissolution and reprecipitation was processed 4 times. Next, the last deposit was stirred up in 30 cc. of water at 0°C, and after agitation with some sodium sulfate, centrifuged in a refrigerated centrifuge. The supernatant was dialyzed against running water (5°C) for 3 days and neutralized to pH 6.8 with 1% NaOH. Precipitation as barium salt and turning into sodium salt was carried out over again. And, the solution containing the sodium salt (30 cc.) was dialyzed, whereby its pH was shifted to 6.0 and its volume increased to 40 cc., chilled to 0°C and added to with 23.6 g. of anhydrous calcium chloride slowly under continuous cooling. The white deposit was filtered off, washed with 59% $CaCl_2$ (pH 6.0) at 0°C and suspended in 30 cc. of 10% NaCl similarly cooled. The suspension was stirred up by means of a homogenizer, and the solution given was dialyzed against running water (about 5°C) for 5 days and precipitated with 3 volumes of alcohol containing 0.05 *M* HCl. Centrifuged. The centrifugate was washed with the alcohol containing hydrochloric acid, 85% and 99% alcohol and ether in turn and dried in vacuo over $CaCl_2$. 58 mg. of a white powder was yielded.

Prep. II. The material started with was "Crude PNA II", which had been obtained as "Crude PNA I" was, employing 0.4 *M* NaCl for tissue extraction. It was submitted to a procedure similar to above, and 163 mg. of Prep. II (a white powder with yellowish hue) was given.

Physical and Chemical Properties of the Preparations

Test-tube tests. Prep. I. Biuret, Goldschmiedt and Neuberg-Saneyoshi negative. Dische (for DNA) almost negative. Molisch, Bial and tests for phosphorus and purine markedly positive.

Prep. II. The tests resulted as in Prep. I, except for Dische test which showed the positive reaction.

Base paper-chromatography of Prep. I. Because of abundant DNA contamination, Prep. II was not examined in this respect.

4.7 mg. of Prep. I was sealed together with 0.5 cc. of 1 *N* HCl in a tube and heated at 100°C (water-bath) for 1 hour, and 0.03 cc. of the hydrolysate was sampled on a sliver of Toyo Roshi filter paper No. 3. The solvent for irrigation was a mixture of tert. butyl alcohol, conc. HCl (sp. gr. 1.18) and water in the volume proportions of 70:8:24. Irrigation ascending. Duration and temperature of test were 50 hours and 25°C respectively. Indication was effected as Masamune and Sakamoto[9] described in the article regarding DNA analysis. As reproduced in Fig. 1, the chromatogram showed spots at the positions corresponding to guanine (Rf 0.30), adenine (Rf 0.46), cytidylic acid (Rf 0.65) and uridylic acid (Rf 0.87).

Composition. The analytical figures obtained are embodied in Table I.

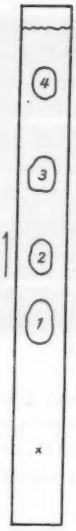

Fig. 1. Base paper chromatogram (2/9×) of the hydrolysate of Prep. I. 1, guanine. 2, adenine. 3, cytidylic acid. 4, uridylic acid.

TABLE I

Composition of Prep. I and II

Analysis	Prep. I		Prep. II	
	in per cent	In ratio	In per cent	In ratio
N*	15.3		14.6	
P†	8.2		7.5	
N/P		1.9		1.9
PNA‡ as yeast PNA E. Merck	95.5		67.7	
DNA§ as human spleen DNA//	4.7		31.0	
Bases¶		Molecular ratios calcd. to a total of 4:		
Adenine	5.7	0.84		
Guanine	11.8	1.56		
Cystosine	17.5	1.11		
Uracil	7.9	0.49		
Purine/Pyrimidine		1.5		
Ash**	4.4		2.4	

* Micro Kjeldahl. † A minor modification of Plimmer method[4]. ‡ Cysteine-sulfuric acid method[5]. § Seibert diphenylamine method[6]. // Converted into Prep. 2 of human spleen DNA of Sakamoto[7]. ¶ A modification of Smith and Markham's method[8]; Kindly analyzed by Dr. M. Sakamoto. ** Pregl method without use of H_2SO_4.

Precipitability. Both the substances are precipitated from water not only by barium acetate and calcium chloride but also by lead acetate and cupric chloride.

Electrophoresis. Either of the preparations showed a single boundary after electrophoresis at pH 7.7 as well as 4.6. See Fig. 2.

Ultraviolet absorption. The absorption spectra of the preparations are plotted in Fig. 3.

Biological Assays

A part of the experiments was carried out by Dr. H. Kawakami in the Department of Obstetrics and Gynecology, Fukushima Medical School and will be published in detail elsewhere.[10] The results including Dr. Kawakami's will be briefly described.

1) Histological changes in liver and kidney of pregnant rabbits after injection of Prep. II. Three pregnant rabbits were injected intraven-

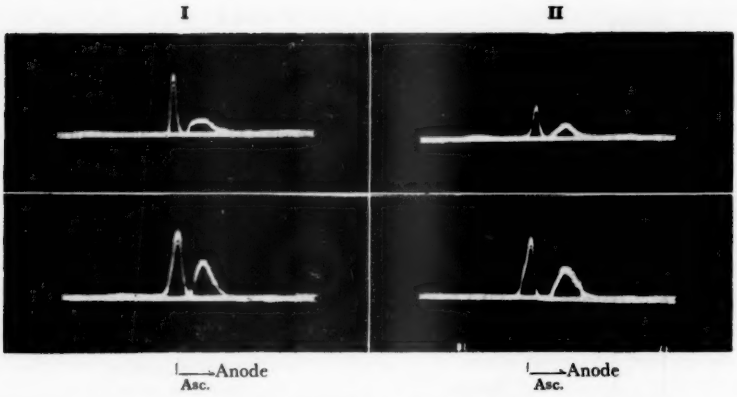

Fig. 2. Electrophoretic patterns (4/5×) of 1% solutions of Prep. I (**I**) and II (**II**) in phosphate buffer pH 7.7, I 0.2 (*a*) and acetate buffer pH 4.6, I 0.2 (*b*) ; current 10 mA. Tested at 17, 15, 18 and 15°C and exposed 30, 22, 21 and 21 minutes after starting current in **I***a*, **I***b*, **II***a* and **II***b* respectively.

Mobility :

$$\mathbf{I}a,\quad \frac{(-1.65)\times 0.007665\times 0.3}{1800\times 0.01}=-21.07\ \text{cm}^2/\text{volt/sec}\times 10^{-5}$$

$$\mathbf{I}b,\quad \frac{(-1.55)\times 0.005678\times 0.3}{1320\times 0.01}=-20.00\quad ,,$$

$$\mathbf{II}a,\quad \frac{(-1.15)\times 0.008291\times 0.3}{1260\times 0.01}=-22.70\quad ,,$$

$$\mathbf{II}b,\quad \frac{(-1.50)\times 0.005470\times 0.3}{1260\times 0.01}=-19.53\quad ,,$$

ously (auricular vein) with 5 mg. per kg. body weight of the substance as a 0.5% solution in physiological saline (The solution was prepared by the aid of NaOH (0.01 *N*). Its pH was 6.8). The animals died 5–24 hours after the injection. The histological findings are illustrated in Table II. Two of the rabbits underwent necrosis and necrobiosis of liver and nephrosis observable in human pregnancy toxemia in distinction from the animals injected with the placental DNA.[2]

2) Effect on blood pressure of pregnant rabbits. Three urethane-narcotized pregnant rabbits were injected with Prep. II in the same dose and manner as in 1). The blood pressure in carotic artery was elevated 8–12 mm. Hg in two of the animals, differing from the case with the placental DNA.[2] In a third animal, it rather lowered as in the control for the DNA experiment by Mugikura.[2] See Table III.

3) Effect of Prep. II on time of pomphus absorption in pregnant rabbits. The assay was processed as described in the foregoing report.[1] As shown in Table IV, the time of pomphus absorption was lengthened

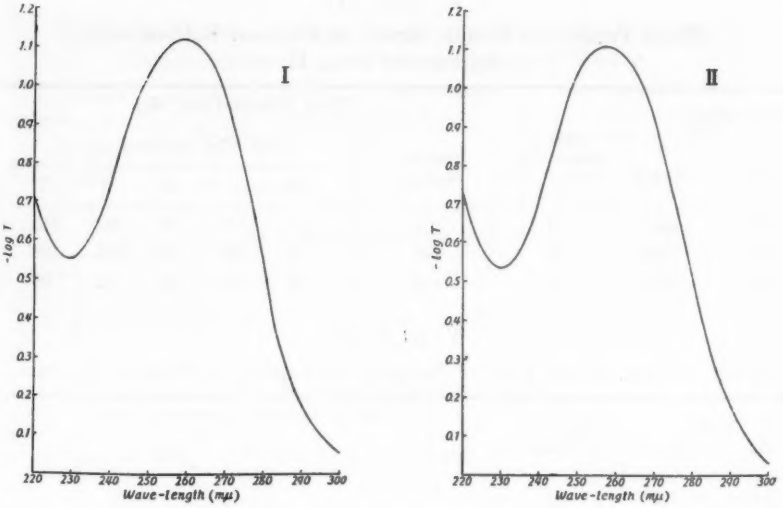

Fig. 3. Ultraviolet absorption spectra of a 0.00475% solution of Prep. I (**I**) and a 0.00492% solution of Prep. II (**II**) in *N*/3000 NaOH. **I**: maximum absorption λ 256mμ, ε(P) 11200; minimum absorption λ 230mμ, ε(P) 5500. **II**: maximum absorption λ 257mμ, ε(P) 11500; minimum absorption λ 230mμ, ε(P) 5300.

TABLE II

Histological Changes in Liver and Kidney of Pregnant Rabbits after Injection of Prep. II

Symbols −∼++ regarding histological changes signify the same as the corresponding symbols in the paper of Kanno *et al.*[11]

Rabbit No.	Rabbit Wt.(g.)	Days of pregnancy	Time of death after inj. (hrs.)	Liver: Necrosis	Liver: Necrobiosis	Liver: Cloudy swelling	Liver: Degeneration	Liver: Stasis of blood stream	Liver: Thrombosis	Liver: Change of Glisson capsule*	Kidney: Nephrosis	Kidney: Glomerulonephrosis	Premature delivery	Aggrav.† of general status after inj.	Albuminuria
142	3000	23	10	+	+	+	+	+	−	−	+	−	−	++	−
143	4500	23	5	−	−	+	−	−	−	−	−	−	−	++	−
144	3000	23	24	+	+	++	+	−	−	−	++	−	−	++	−

* Cellular infiltration and swelling of the sheath. † ++: strong aggravation.

TABLE III

Blood Pressure in Carotic Artery of Pregnant Rabbits after Injection of Prep. II

Rabbit No.	Rabbit Wt.(g.)	Days of pregnancy	Blood pressure (mm. Hg) Before injection	Time after injection (min.) 0.5	1	3	5	7
145	3000	23	98	95	93	97	102	110
146	4500	23	100	98	98	98	102	108
147	3000	23	100	98	92	90	92	98

TABLE IV

Effect of Prep. II on Time of Pomphus Absorption in Pregnant Rabbits

Rabbit No.	Rabbit Wt.(g.)	Days of pregnancy	Time of pomphus absorption (min.) Before injection of Prep. II	After the injection
148	3000	23	7	6
149	4500	23	$6^1/_2$	$7^1/_3$
150	3000	23	5	$7^1/_6$

in two of three animals used, and shortened in the remaining.

4) No albuminuria occurred in any of the animals in Table II.

5) No positive precipitin reaction occurred at all, when 1% and progressively diluted solutions of Prep. II in physiological saline were layed over sera of non-pregnant, normal-pregnant and pregnancy-toxemic women. See Table V.

6) Skin reaction. The assay was processed also as described in the foregoing article,[1] employing Prep. I, and the results in Fig. 4 were given. Normal-pregnant, puerperal, pregnancy-toxemic and particularly cancer-suffering women looked to react more intensively than the non-pregnant.

SUMMARY

1. Pentosenucleic acid was prepared from human placentas in electrophoretically homogeneous state. Prep. I was contaminated only with less than 5% DNA, but Prep. II with more than 30% DNA. They were investigated not only chemically but in biological connection with pregnancy-toxemia.

2. Prep. II caused histological lesions in liver and kidney and elevated blood pressure, when injected intravenously into pregnant rabbits. It also prolonged the time of absorbing a blister produced by subcutaneous injection of physiological saline in pregnant rabbits. Prep. I showed

TABLE V

Precipitin Reaction of Prep. II with the Sera of Non-pregnant, Normal-pregnant and Pregnancy-toxemic Women

Symbol — signifies no occurrence of the reaction.

No. of individual	Dilution of the antigen (1:)				Symptoms of patients		
	10^2	2×10^2	3×10^2	4×10^2	Blood pressure (mm. Hg.)	Albumi-nuria	Edema
Sera of non-pregnant women							
1	—	—	—	—			
2	—	—	—	—			
3	—	—	—	—			
4	—	—	—	—			
5	—	—	—	—			
Sera of normal-pregnant women							
6	—	—	—	—			
7	—	—	—	—			
8	—	—	—	—			
9	—	—	—	—			
10	—	—	—	—			
11	—	—	—	—			
Sera of pregnancy-toxemic women							
12	—	—	—	—	156-100	(++)	(+)
13	—	—	—	—	136- 90	(+++)	(+)
14	—	—	—	—	120- 90	(−)	(+)
15	—	—	—	—	158- 90	(+)	(++)
16	—	—	—	—	150- 90	(+)	(++)
17	—	—	—	—	160-100	(+)	(++)

tendency of giving the positive skin reaction for pregnancy and cancer.

3. Placental PNA must be a strong factor inducing pregnancy toxemia like placental proteins of which Yosizaki will inform later on.[3]

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2) Mugikura, *ibid.*, 1955, **61**, 261.
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4) Haneda, Sinokawa & Yasuoka, *ibid.*, 1944, **46**, 263.
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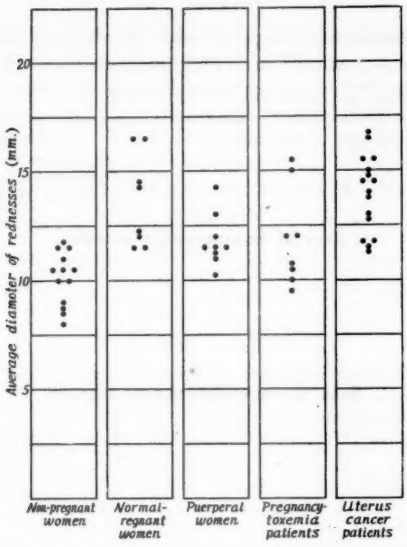

Fig. 4. Showing skin reaction of Prep. I in non-pregnant, normal-pregnant and puerperal women and pregnancy-toxemia- and cancer-patients.

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Excitation and Initiation of Impulse

Part I. Stimulation with Constant Current

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General Introduction and Way of Approach

In investigations hitherto made on the law of excitation, it has always been the excitation at the stimulated locus that was considered in theories, while it has been the propagated impulse that was employed as index of excitation in experiments. This might have been a source of confusion, because excitation and conduction are not always identical.

Rushton[1], later Yamagiwa[2),2a)], claimed that a certain minimal length L of a fibre must be excited for initiation of impulse. According to them, an excitation of a fraction of L remains localized or conducted just locally, although graded in size of potential or of conducted distance, according to the size of the fraction excited. Whether this genuine "local response" is identical or not with the so-called "subthreshold activity" (see Stämpfli[3)]), is a problem[2)], but it is a different matter here.

Now, if such a length L really exists, then simple excitation is concerned with a "point", while initiation of impulse with simultaneous excitation of the whole L. From this consideration, theoretical treatments can be developed on various experiments concerning initiation of impulse. In the present argument, eq. (1) (Fig. 1) derived by myself recently[2),2a)] was adopted as the quantitative basis of L, when necessary:

$$y = k \ln f\left(1 - e^{-\frac{x}{k'}}\right) \qquad (1)$$

x: active (excited) length,
y: resting length to be activated by x,
k, k′: length constants of resting and active membranes,
f: safety factor for local excitation ($=\frac{V}{S}$, V peak value of action potential and S threshold in terms of V reduced properly).

Assume for simplicity that the stimulating current spreads in one direction only, and pay attention to the point L (Fig. 1) were y=x. The

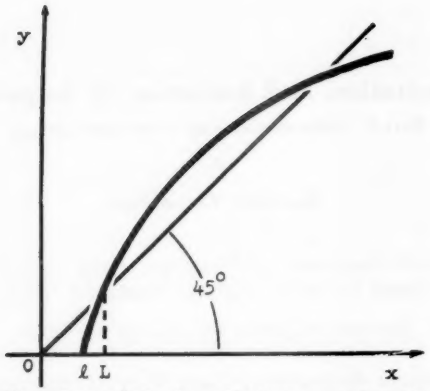

Fig. 1. Normal y-x-relation in squid giant axon, schematic. $k \fallingdotseq 5$ mm ; $l \fallingdotseq 0.1$ mm ; $L \fallingdotseq 0.11$ mm ; $f \fallingdotseq 10$ (assumed).

length OL (simply L hereafter) represents the minimal length above mentioned. Now, the current I_L at x=L must be weaker than I_0 at x=0 where the electrode is set, because $I_L = I_0 e^{-\frac{L}{k}} = \frac{I_0}{m} (m = e^{\frac{L}{k}} > 1)$. There must be some time difference Δt between the moments of excitation, t_0 and t_L, of the two points. The necessary condition for propagation is then practically $\Delta t = t_L - t_0 \leqq D$, D being the duration of excitation at x=0. Our task is to evaluate t_L and t_0 and equate $\Delta t \leqq D$, to examine the results therefrom.

A rough examination was attempted previously[2]. The present paper aims at its completion, revising and extending it much farther. Figures and notations were changed in part. Simple repetitions were avoided except of essential points.

I. i_r, I_r and t_r.

Eq. (2) which has appeared repeatedly in the literature[4]-[7] was chosen as a representative of the law of excitation :

$$I = \frac{i_r}{1 - e^{-\frac{t}{\alpha}}} \qquad (2)$$

I : applied current,
i_r : rheobase for excitation (for $t=\infty$),
α : time constant proper to nerve ($=k_{(H)}$, Hill's constant of local potential).

This equation yields I_r (I for $t=\infty$) $=i_r$, which means that the applied current itself becomes identical with i_r, in the extreme case. However, the matter appears quite different if viewed from the present theory, as shown below :

At points x=0 and x=L respectively,

$$I=I_0=\frac{i_r}{1-e^{-\frac{t_1}{\alpha}}};\ I=I_L=\frac{I_0}{m}=\frac{i_r}{1-e^{-\frac{t_2}{\alpha}}},$$

which give

$$t_1=\alpha\ln\frac{I_0}{I_0-i_r};\ t_2=\alpha\ln\frac{I_0}{I_0-mi_r} \tag{3}$$

Hence, $\Delta t=t_L-t_0=(t_2+\theta_2)-(t_1+\theta_1)=\alpha\ln\frac{I_0-i_r}{I_0-mi_r}+(\theta_2-\theta_1)$

$\theta_{1,2}$: latencies at x=0 and x=L.

θ must be a function of intensity and duration of the pulse[8], but m being very near unity as will be stated later, and accordingly I_0 and I_L very near each other in normal cases, $(\theta_2-\theta_1)$ will be small enough to be neglected against $\alpha\ln\frac{I_0-i_r}{I_0-mi_r}$, at least when I_0 is small. The condition for propagation is then

$$\Delta t=\alpha\ln\frac{I_0-i_r}{I_0-mi_r}\leqq D \tag{4}$$

I_r, the smallest value of I_0 which satisfies eq. (4), that is, *the rheobase for propagation*, is

$$I_r=i_r\frac{me^{\frac{D}{\alpha}}-1}{e^{\frac{D}{\alpha}}-1}=i_rm\{1+\frac{m-1}{m}\frac{1}{e^{\frac{D}{\alpha}}-1}\}=m\eta i_r \tag{5}$$

$$\eta=1+\frac{m-1}{m}\frac{1}{e^{\frac{D}{\alpha}}-1}>1.$$

In case $i_r<I_0<I_r$, a localized excitation will take place in the vicinity of the point x=0.

Introducing eq. (5) into (3),

$$t_2=\alpha\ln\frac{m\eta i_r}{m\eta i_r-mi_r}=\alpha\ln\frac{\eta}{\eta-1}$$

This t_2 represents t_r, the so-called " Hauptnutzzeit ", because t_2 in general is nothing but the utilization time being explored in experiments. Hence,

$$t_r=\alpha\ln\frac{\eta}{\eta-1} \tag{6}$$

Eq. (5) expresses that the current flowing at x=0 in the so-called rheobasic stimulation is $m\eta$-times, and the current at x=L $(=\frac{I_r}{m})$ is η-times larger than i_r. The value of η depends upon m, D and α. In frog's motor nerve, we may put $D\fallingdotseq 1$ msec, and $\alpha\fallingdotseq 0.5$ msec in average, so

$$\eta \fallingdotseq 1+\frac{m-1}{m}\frac{1}{e^2-1} \fallingdotseq 1+\frac{m-1}{m}\frac{1}{7}$$

The normal value of m is estimated to be about 1.02 as will be shown in Part II, hence $\eta \fallingdotseq 1.003$ and $\frac{\eta}{\eta-1} \fallingdotseq 3\times 10^2$. Then, putting $\eta \fallingdotseq 1$ in eq. (5), we have $I_r \fallingdotseq m i_r \fallingdotseq 1.02\ i_r$, and from eq. (6), $t_r \fallingdotseq 0.5\times 2.3\times 2.5 \fallingdotseq 2-3$ msec. We see thus that 1) I_r is larger than i_r by a few %, and 2) t_r is as large as a few msec, which is just about the value actually obtained. It is important to remember here that I_r is the *rheobase for propagation*, and t_r the utilization time of $\frac{I_r}{m}$ flowing at $x=L$.

In the previous paper[2], it was shown that Weiss' and Nernst's equations give similar results, too. Other equations will do the same, provided that I as a function of t yields gradually larger Δt for gradually smaller I.

II. Repetitive responses

Nerve fibres are known to respond repetitively to long-lasting constant current. The characteristics are said to be considerably variable, but the typical ones seem to be the following three : 1) larger frequencies in stronger stimuli, 2) gradual decline of frequency with time, and 3) cessation of the response after a certain time. If assumed that each response does not disturb the local processes, then the cessation of the response is comprehensive from the theory of accommodation as the result of i_r-rise up to the level of the local potential evoked (Katz[9]), but the other two are not always so, because, the local potential being assumed to remain fixed after once evoked, the response interval should be determined solely by the refractory period.

If viewed from the present theory, whether the response is repeated or not, depends upon whether eq. (4) is established or not for new Δt after the first response. One possible occurrence may be a gradual rise or i_r or m with time, which makes $t_{1,2}$ (eq. (3)) and Δt (eq. (4)) gradually larger. Leaving this to be dealt with in Part III, we will consider here another possibility, i.e., the gradual rise of α. This is a speculation at present, but seems fairly probable as a result of gradual reduction of the membrane polarizability, probably taking place in repeating the responses. If it occurs, then it, too, enlarges both $t_{1,2}$ and Δt. Gradually larger t_2 will result in gradual prolongation of the response interval, and gradually larger Δt in cessation of the response at the moment where $\Delta t=D$ is reached. The higher frequency in stronger I_0 is shown in eq. (3) (for t_2) itself. The three typical features can thus be well understood, without introducing "accommodation" although just qualitatively.

Important findings have been made by Katz[9] and Sato[10] that fibres with small β_r (the liminal gradient, see Part II) or large λ (Hill's constant of accommodation) are liable to give rise to repetitive responses. In the present theory, $\beta_r=\frac{I_r(m-1)}{\eta Dm}$ (Part II, eq. (1)) and $\lambda=\frac{Ir}{\beta_r}=\frac{\eta Dm}{m-1}$. Small β_r means therefore small I_r, small m (>1) and large D. Large λ also means small m and large D. Evidently, large D makes the establishment of the condition $\Delta t \leqq D$ easier. Small i_r and m (accordingly I_r) do the same by reducing Δt. Katz' and Sato's findings can thus be explained satisfactorily. Sato's[11] and Diecke's[12] observations that a larger Na$^+$-concentration of the bathing fluid makes β_r smaller and at the same time repetitive responses easier, are very interesting in this connexion.

Discussion

1) Existence of L. The length L is not yet verified experimentally because of methodical difficulties, but the possibility is suggested also by an ideal experiment, as follows:

Suppose a fibre separated into halves by a wall of zero thickness, and the surrounding medium shunted over the wall with a break-key in the circuit. Excite the fibre at its one end, and break the key shortly after the impulse has reached the wall. If the time is short enough, the impulse would not propagate beyond the wall, because there must be some utilization time as regards the action current as stimulus. Denote the critical time by Δt and the conduction velocity measured separately by v. The quantity $v \times \Delta t$ represents the length which has been travelled (activated) during Δt by the impulse. The actual cause of interruption must have been that $v \times \Delta t$ was not large enough for evoking further propagation.

2) Strength-duration relation in initiation of impulse. 1) Current and time to be related. Eq. (2) may be granted as a law of local excitation. But, as stated above, the time t explored in experiments employing propagated impulses as indices, is nothing but t_2 (eq. (3)), the utilization time of $\frac{I_0}{m}$ flowing at $x=L$. Therefore the obtained t should be related to $\frac{I_0}{m}$, but not to I_0, 2) The region of $I_0<I_r$ and $t>t_r$. As long as eq. (2) is correct, I_0 weaker than I_r (but stronger than i_r) and t larger than t_r must also be effective for eliciting a local excitation. But as far as initiation of impulse is concerned, I_r and t_r represent respectively the weakest current and the longest time available, and, therefore, the point (I_r, t_r) in the strength-duration-curve marks the termination of the curve, theoretically as well as practically. The continuation of the curve might be

drawn horizontal, but it has no significance. 3) Eq. (3) as the proper expression of the relation. From 1) and 2) above, it follows necessarily that the theoretical strength-duration-relation in experiments employing propagated impulses as indices should be expressed by eq. (3), not eq. (2), in combination with a limitation in t, as follows:

$$t_2=\alpha \ln \frac{I_0}{I_0-mi_r},\ t_2\leqq t_r.$$

Or, taking the original form, $\frac{I_0}{m}=\frac{i_r}{1-e^{-\frac{t_2}{\alpha}}}$, and multiplying by ηm, and replacing t_2 by t,

$$\eta I_0=\frac{I_r}{1-e^{-\frac{t}{\alpha}}},\ t\leqq t_r\ \left(=t_{I_0=I_r}=\alpha \ln \frac{\eta}{\eta-1}\right)$$

This equation is scarecely different from eq. (2), but there are two points to be noticed, i.e., I_r and $t\leqq t_r$. I_r, the rheobase for propagation, is a certain definite quantity which can be caught experimentally, in contrast to i_r which is imaginative. The limitation $t\leqq t_r$, excluding the region of $t>t_r$, makes us free from the ambiguities and uncertainties which we have encountered in theoretical considerations of the whole range of t in connexion with i_r.

3) I_r and t_r. Hill's interpretation[4] of the so-called rheobase and its utilization time fails when no accommodation occurs. Hashida[13] made distinction between theoretical (for $t=\infty$) and experimental rheobases, and took t_r as the utilization time of the latter. This made the matter simpler and clearer, but still it was strange, as well as suggestive, that a slight reduction of the applied voltage makes the stimulus totally ineffective, or, so to speak, the utilization time jump from t_r abruptly to ∞.

I_r in the present theory might appear to correspond to Hashida's experimental rheobase, and t_r to its utilization time. But in the present argument, the appearance of I_r and t_r of certain definite values is a matter of theory, not simply of practice, expected to occur *in the extreme case*. The abrupt jump of t_r is in actuality an expression of $\Delta t>D$, and does never mean a true elongation of t_r. The point is that I_r refers to the point $x=0$ and t_r to the point $x=L$.

4) I_r and i_r. The existence of i_r seems highly probable, but as long as the present theory is correct, it will never be detected by employing propagated impulses as indices, because what is caught in this way is I_r and not i_r.

5) τ (chronaxie)-α- and τ-t_r-relation. If α is replaced by $k_{(H)}$ (Hill's constant of local potential), eq. (2) turns out Hill's equation itself. There-

fore, τ-α-relation is nothing but τ-$k_{(H)}$-relation in Hill.

Now, τ-α-relation, if derived directly from eq. (2), is

$$\tau \text{ (t for } I=2\ i_r) = \alpha \ln 2,$$

which is claimed in Hill as a general relation between τ and $k_{(H)}$. But if derived from the present theory (eq. (3)),

$$\tau \text{ (t_2 for } I_0=2\ I_r) = \alpha \ln \frac{2m\eta i_r}{2m\eta i_r - m i_r} = \alpha \ln \frac{2\eta}{2\eta-1} \qquad (7)$$

In case $\eta \fallingdotseq 1$ as in normal frog's motor nerve, τ can be equal to $\alpha \ln 2$, but not always so in other cases, because η depends upon m, D and α.

Further, a general theoretical relation between τ and t_r can be derived from eq. (6) and (7):

$$\frac{t_r}{\tau} = \ln\frac{\eta}{\eta-1} / \ln\frac{2\eta}{2\eta-1} \qquad (8)$$

If applied to the normal frog's motor nerve, employing the approximate value of η above estimated, we obtain

$$\frac{t_r}{\tau} \fallingdotseq \frac{\ln 300}{\ln 2} \fallingdotseq 8,$$

which agrees well with Sakamoto's data[14] in average, where t_r was 1.03–1.24 msec and τ 0.04–0.246 msec.

6) Repetitive responses. Katz's interpretation was criticized above. Tasaki[15] also treated the problem from his own theory. He assumed that, after the first response, the rheobase returns back from ∞ to a new steady level β_0 following the equation $\beta(t)=\beta_0 e^{\frac{1}{\kappa t}}$, κ being a constant; further that the next response takes place after a time T where $\beta_0 e^{\frac{1}{\kappa T}} = v$ (applied voltage). The equation obtained was $\frac{1}{T}$ (=F, frequency) $= \kappa \ln \frac{v}{\beta_0}$. He predicts further that one of the necessary conditions for excitation, $\frac{dE_s(t)}{dt} \geqq \frac{1}{\lambda} E_s(t)$, the "excitatory state"), will become unfulfilled sooner or later because of λ being reduced. Thus, the rise of F with v, and the cessation of response after a certain time could be well explained. The gradual fall of F is comprehensive, too, if introduced the gradual rise of β_0. The difference is that repetition of excitation is considered in Tasaki, while repetition of impulse initiation in the present theory. Another point is that Tasaki's equation as it is pionts to infinite rise of F with v, which is evidently unreasonable because there exists a certain absolutely refractory period. Just the first response, which he did not treat, is free from this, so that, if viewed from the present theory, the response time can be expressed prac-

tically by t_2 of eq. (3). In this case, we have $I_0\ (e^{\frac{t_2}{\alpha}}-1)=mi_r e^{\frac{t_2}{\alpha}}$, from which $\frac{I_0}{mi_r}(=\frac{\eta I_0}{I_r})=\frac{e^{\frac{1}{\alpha F}}}{e^{\frac{1}{\alpha F}}-1}$ $(F=\frac{1}{t_2})$. Putting $e^{\frac{1}{\alpha F}}\fallingdotseq 1+\frac{1}{\alpha F}$, excepting the range of small αF, we have

$$\frac{\eta I_0}{I_r}=\alpha F\ (\frac{1}{\alpha F}+1)=1+\alpha F,\quad F=\frac{1}{\alpha}(\frac{\eta I_0}{I_r}-1)\ \text{(Fig. 2, I)}\quad (9)$$

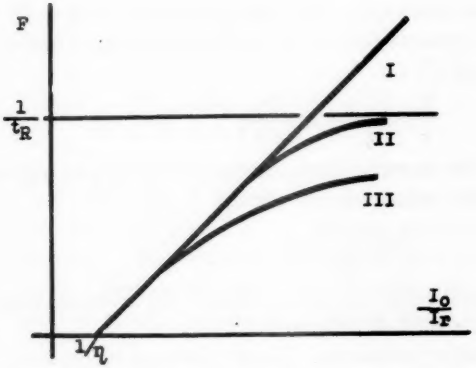

Fig. 2. $F-\frac{I_0}{I_r}$-relation. F: Frequency. t_R: Abs. ref. period. I, II, III: See text.

For the 2nd response, however, F should be determined by $\frac{1}{t_2+t_R}$, where t_2 represents a new response time and t_R the refractory period. The effect of t_R is relatively little in the range of large t_2 (that is, small I_0), but predominant in the range of small t_2 (that is, large I_0). Therefore, F will depend largely upon t_2 in weak I_0 and largely upon t_R in strong I_0, so that the F-$\frac{I_0}{I_r}$-curve will run practically linear in some initial stage, almost in coincidence with the curve I, but later bend downwards to attain $F=\frac{1}{t_R}$ in the end (Fig. 2, II). The same thing can be said about the 3rd and following responses, but every curve is expected to run somewhat below that of the preceding response (curve III), because α and also I_r (see Part III) may increase gradually. This was found really the case in Sato[10)-11)].

The above expectation agrees well with Hodgkin's statements[16)] about the response of *Carcinus* axons (Class 1) that 1) " response time rather than

refractory period is the primary factor in determining the frequency of repetition when the current is weak ", and 2) " the refractory period became important when the current strength was large and the repetition frequency was high. Strong currents reduced the response time towards zero but the repetition interval never became less than 6.5 msec.—— ". The significance of the length of the response time was interpreted in terms of the duration of the " local response ", the electric sign associated with it, but it is a different matter.

Hodgkin made many other interesting observations, of which some of particular importance will be quoted and discussed below: 1) In axons belonging to Class 1, which could respond over a wide range of frequencies (5–150/sec), " the local subthreshold potentials were small compared to the propagated action potentials ", and further " the response time was extremely long when the strength of current was weak." The former points to small m, because ,in the present view[2], the so-called subthreshold potential just necessary for evoking propagation represents the potential due to excitation of just L; the smallness of the value can therefore mean small L, that is, small m. The latter, too, does the same, because it means practically a very long t_r, which means in turn that η (>1), accordingly m (>1), is very near unity (see eq. (6)). Such a small m makes t_2 and Δt small, by which the repetitive response will be maintained long, with frequencies declining with time owing to gradual rise of α or also of m, as will be stated in Part III. 2) Axons with pronounced supernormal phase (Class 2) gave trains of impulses of " fairly high " frequencies, which " might last for several seconds but which ended abruptly without giving frequencies lower than 50/sec." In view of the present theory, supernormality means small I_r $(=m\eta i_r)$, which can mean again small m. Roughly, therefore, the repetitive response may be considered as taking place in a state of reduced m. This makes the high frequency and the long duration of the responses comprehensive. However, the abrupt termination without passing the stage of lower frequencies cannot be explained by such a simple assumption. The matter must be much more complicated in actuality. 3) Axons diminish the ability to repeat if left in oil or sea-water for a long period (Class 3). Under these circumstances, " the response time at rheobase is greatly reduced—— ", and " the rheobase itself had increased considerably." The latter facts indicate an enlargement of m, which, contrary to the case 1) above, makes the repetitive response difficult or impossible. 4) Subthreshold oscillations were observed in just subthreshold stimulations (Text—Fig. 6, I–J), which " appeared to differ in several respects from the oscillations seen in decalcified nerves or in nerves stimulated with strong currents." They seemed to occur " over a very narrow range of currents " and " when the axon just fails to propagate." Such oscillations are of a

particular significance in view of the present theory, because currents lying between I_r and i_r are to elicit repetitive local excitations of relatively low frequencies.

Sato[10] developed recently a new mathematical treatment of the repetitive response, based on Tasaki's[15] theory of excitation and on the idea of accommodation, and was led to the same equation as Katz's[9]. Katz's equation, however, represents just a special case of i_r-rise if viewed from the present theory as will be stated in Part III. One of Sato's[11] observations worthy of special note is that a node of Ranvier responds repetitively only rarely if stimulated directly, even when immersed in a 5% NaCl solution. It is not quite clear at present what this fact signifies, but it might be a clue for clarifying a part of the mechanism of the repetitive initiation of impulse.

Summary

1. Standing on a theoretical ground, excitation was distinguished from initiation of impulse.

2. Regarding the initiation of impulse as due to simultaneous excitation of a certain length L in less than D (duration of excitation), a theoretical treatment was developed on various phenomena of excitation. Chief phenomena explained were, a) appearance of I_r (rheobase for initiation of impulse) combined with a proper duration, t_r, and b) repetitive responses without rise of threshold.

3. Discussions were made on a) existence of L, b) strength-duration-relation in initiation of impulse, c) I_r, i_r, t_r and τ (chronaxie), and 4) repetitive responses.

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Excitation and Initiation of Impulse

Part. II. Stimulation with Slowly Rising and Alternating Currents

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In this Part, it will be shown that 1) the liminal gradient β_r in stimulation with linearly rising current, 2) the linear relationship between the final current and the time constant in stimulation with exponentially increasing current, and 3) the optimum frequency in stimulation with alternating current, can be the case without rise of threshold, that is, independent of accommodation in the classical sense.

I. Stimulation with Linearly Rising Current

Employing nerve trunks as material, Lucas[1] found 1) gradual rise of threshold with decrease of the gradient, and 2) a certain liminal gradient, β_r, below which no impulse can be fired off. Recently, contradicting results were obtained by Sugi[2] in skeletal muscle and by Tasaki[3], Frankenhäuser[4] and Diecke[5] in single nodes of Ranvier, who all observed threshold constancy in a wide range of the gradient. The gradual rise in Lucas was ascribed to complications associated with nerve " trunks " by Tasaki, and to distortion of the stimulating current due to polarization of the epineurium and others by Diecke. At any rate, it is note-worthy that the rise almost disappeared with appearance of modern methods of fibre isolation and electric insulation. Here, we pay attention to the constancy of threshold, leaving the rise observed near β_r[3)-5)] to be discussed in Part III.

1. β_r

Suppose I_0 (and I_L) converted into a constant current at the level of I_r (and $\frac{I_r}{m}$). Referring to Fig. 1,

$$\Delta t = t_L - t_0 = (t_2 - t_1) + \varepsilon, \quad \varepsilon = t_{N_2} - t_{N_1}.$$

Strictly, t_N must be a function of the gradient, but I_0 and I_L being very

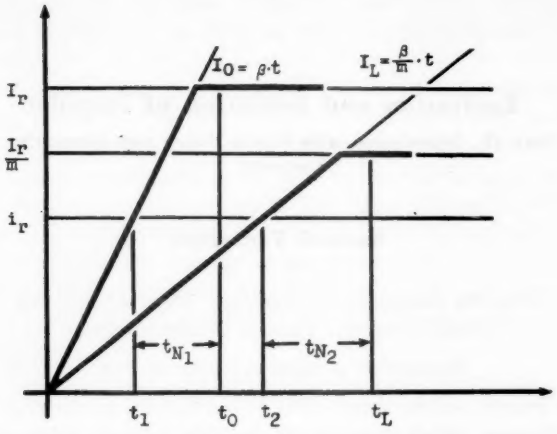

Fig. 1. Stimulation with linearly rising current. I_0, I_L: currents at x=0 and x=L, respectively. i_r, I_r: rheobases for excitation and propagation, respectively. $t_{1,2}$: moments at which I_0 and I_L reach i_r respectively. t_0,t_L: moments of excitation at x=0 and x=L, respectively. $t_{N1,N2}$: times (including latencies) required for excitation after $t_{1,2}$ respectively.

little different, t_{N1} and t_{N2} may be regarded to be so, too, in normal conditions. So, neglecting ε against (t_2-t_1),

$$\Delta t=\frac{mi_r}{\beta}-\frac{i_r}{\beta}=\frac{i_r}{\beta}(m-1),$$

i_r, m: constant (assumed).

The limiting condition for propagation* (see the note in "References and Notes") is $\Delta t=D$, hence

$$\beta_r=\frac{i_r(m-1)}{D}=\frac{I_r}{\eta D}\frac{m-1}{m} \tag{1}$$

If we denote the standard values of η, i_r, I_r, m, D and β_r by η_0, $i_{r\cdot 0}$, $I_{r\cdot 0}$, m_0, D_0 and $\beta_{r\cdot 0}$ respectively, then

$$\beta_{r\cdot 0}=\frac{i_{r\cdot 0}(m_0-1)}{D_0}=\frac{I_{r\cdot 0}}{\eta_0 D_0}\cdot\frac{m_0-1}{m_0} \tag{2}$$

In the case of nodal membrane, we may take $D_0\fallingdotseq 1$ msec. (D of a whole node may be prolonged in stimulation with small gradients as suggested by the delayed peak observed by Diecke[5]. But this is different from D at x=0, because a whole node can be put into action in various lengths of time according to β, if viewed from the present theory.) Further, we may represent $I_{r\cdot 0}$ by the voltage effective at the edge of a nodal membrane where $I_{r\cdot 0}$ flows in rheobasic stimulation. This is about 30/3 mV because

the rheobase in node-to-node stimulation is about 30 mV[10] and the nodal membrane resistance is about equal to the internodal axonal. The only unknown quantity m_0 may be roughly estimated in the following way: First assume $L \fallingdotseq l$ (see Part I, Fig. 1 and legend), and $f \fallingdotseq 10^{6),7)}$. Now, l (x for y=0) $\fallingdotseq 0.1$ k', hence $\frac{L}{k} \fallingdotseq 0.1 \frac{k'}{k} \fallingdotseq 0.1 \sqrt{\frac{\gamma_m'}{\gamma_m}}$, $\gamma_{m,m}'$ being the resting and active membrane resistances of unit length of fibre. According to Tasaki and Freygang[8], $\gamma_m' < \frac{\gamma_m}{10}$, hence $\frac{L}{k} < \frac{0.1}{3}$, accordingly $m_0 = e^{L/k} < e^{0.03} \fallingdotseq 1.03$. This figure is very close to that of a squid giant axon where $m_0 \fallingdotseq e^{0.1/5} \fallingdotseq 1.02$ (Part I, Fig. 1, legend). So, taking $m \fallingdotseq 1.02$ and $\eta \fallingdotseq 1$, we have $\beta_{r.0} \fallingdotseq \frac{10 \times 0.02}{1.02} \fallingdotseq 0.2$ mV/msec. at the stimulated membrane, or $\beta_{r.0} \fallingdotseq 0.2 \times 3 \fallingdotseq 0.6$mV/msec. in the voltage applied, which is in fair agreement with Tasaki's observation[9] where β_r was 0.1–1.0 mV/msec. with respect to the applied voltage.

Eq. (5) in Part I and eq. (1) in this Part show respectively that a small change of m brings about a small change of I_r and an enormous change of β_r. This provides a probable reason for the larger values of I_r and β_r reported by Diecke[5] ($I_r \fallingdotseq 40$ mV (Abb. 7 and 13); $\beta_r = 0.8$–6 V/sec), as compared with those of Tasaki, where I_r was mostly a little over 30 mV[10], and β_r was 0.1–1.0 mV/msec. as stated above. For, if m is 1.2 instead of 1.02, then I_r should be larger by about 20%, while β_r by about 10-times. This is satisfactory for explaining the discrepancy between Tasaki and Diecke. Why m was so large in Diecke's preparations is not clear, but the matter might be comparable with that of *Carcinus* axons left in oil or seawater for a long period (see Hodgkin's work quoted and discussed in Part I, Discussion).

2. Repetitive Responses

Repetitive responses to linearly rising current have been investigated by several authors, for example by Skoglund[11] and Granit and Skoglund[12] in recent years. A theoretically very important one is that of Hoffmann[13] that nerve continues to respond repetitively as long as a current with a superthreshold gradient continues to increase. An explanation was given by Katz[14] on the basis of Hill's accommodation, but thereby the effect of each responses upon the local processes were disregarded. An explanation from the present theory is as follows: Denote the moment at which I_0 reaches i_r by t_0 (Fig. 2), the interval between t_0 and the first response at x=0 by t_1, and the interval between t_1 and the second response by t_2, and so on.

The stimulating current for the n-th response supposed to occur is

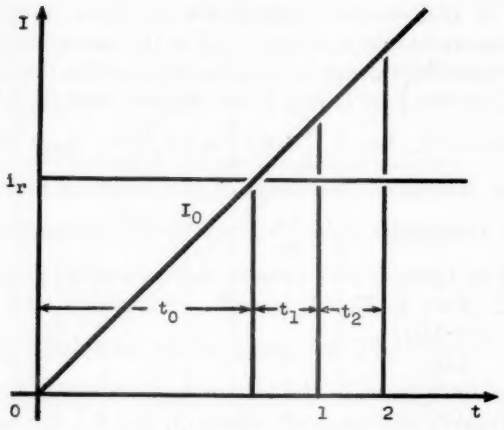

Fig. 2. Repetitive responses to linearly rising current. Abscissa: No. of responses supposed to occur. About details, see text.

expressed by $I_{0,n}=\beta\ (t_0+t_1+-----+t_{n-1})+\beta t$
$=i_r+\beta T_{n-1}+\beta t,\ T_{n-1}=t_1+-----+t_{n-1}$.
The constant component $(i_r+\beta T_{n-1})$ becomes more and more while the linearly rising part βt less and less prominent with time. If we disregard the latter for simplicity, then the whole picture turns into that of a special case of constant current stimulation, where the current intensity is made to jump stepwise after each response.

The same thing can be said about the point x=L, the only difference being that the current is $\frac{I_0}{m}$. Then, the n-th Δt for the n-th response, supposed, in the case of $\beta=\beta_r$, can be obtained from eq. (4) (Part I) by putting $I_0=i_r+\beta_r T_{n-1}$:

$$\Delta t_n=\alpha \ln \frac{i_r+\beta_r T_{n-1}-i_r}{i_r+\beta_r T_{n-1}-mi_r}=\alpha \ln \frac{\beta_r T_{n-1}}{\beta_r T_{n-1}-(m-1)\,i_r}$$
$$=\alpha \ln \frac{1}{1-\frac{(m-1)i_r}{\beta_r T_{n-1}}}=\alpha \ln \frac{1}{1-\frac{D}{T_{n-1}}}\ \text{(eq. (1) introduced)}\quad (3)$$

T_{n-1} gets larger with increase of n; as a consequence, Δt_n gets smaller, by which the condition for propagation $\Delta t \leqq D$ is maintained on without fail. Eventually, Δt may rise temporarily as the result of α-rise, but this effect can be suppressed by raising β properly. We see after all that nerves will persist to respond repetitively if β is chosen larger than β_r in proper magnitude. As to possibilities of i_r- and m-rises, see Part III.

3. Consideration

1) Physiological meaning of β_r. In Hill's theory of accommodation[15], the threshold is predicted to attain ∞ at β_r, after gradual rise. In actuality, however, it was found finite, that is, 1.2–1.3 times normal[3)–5)]. Another serious fact is that a slight reduction of the gradient makes the current totally ineffective, that is, that the threshold becomes " ∞ " suddenly, which is not easy to understand.

Diecke[5] interpreted β_r as " die Zeitkonstante für die Inaktivierung der Na^+-Permeabilität und Aktivierung der K^+-Permeabilität der Membran durch unterschwellige Vordepolarisation ", and emphasized that " die Akkommodation (im Sinne Hill's) ein Artefakt am Nervenstamm ist." Yet, the inefficacy of gradients smaller than β_r was explained, as in Hill, in terms of threshold, which, he found, continued to rise up almost exponentially, to escape from the stimulating current. Tasaki[9),10),16)] regarded β_r as the threshold gradient necessary for "'excitation " in stimulation with slowly rising current in general. In the present theory, however, β_r, being conditioned by $\Delta t \leqq D$, appears as a requisite for " initiation of impulse ", not for excitation, and the inefficacy of smaller gradients, being conditioned by $\Delta t > D$, has nothing to do with the threshold.

2) β_r/I_r and I_r/β_r. These quantities were adopted by Lucas[1] and Fabre[17] to characterize the excitability. They correspond respectively to $\frac{m-1}{\eta D m}$ and $\frac{\eta D m}{m-1}$ in the present theory (see eq. (1)). Hill's constant λ, which is nothing but $\frac{I_r}{\beta_r}$, is therefore a function of m, too, as I_r is so.

3) β_r and temperature. In low temperature, I_r is said to change very little, while β_r gets very small[9] : a fact well comprehensive as due, in part, to prolongation of D (see eq. (1)). As to a possibility of fall of m, see Part III.

II. Stimulation with Exponentially Increasing Current

1. Linear relationship between final current and time constant

An exponentially increasing current is expressed by

$$I = I_\infty \left(1 - e^{-\frac{t}{\tau}}\right)$$

I_∞ : final current,
τ : time constant.

Recent investigations in this field have revealed that impulse is released at an almost constant level of potential[2),5)], irrespective of the rate of increase of the current. Let us examine the problem from the present theory,

assuming i_r and m constant.

Referring to Fig. 3, we have

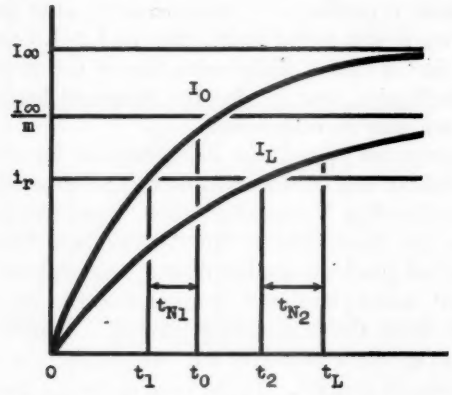

Fig. 3. Stimulation with exponentially increasing current. Notations mean the same as in Fig. 1.

$$\Delta t = t_L - t_0 = (t_2 - t_1) + (t_{N2} - t_{N1})$$

Neglecting the term $(t_{N2}-t_{N1})$ for the same reason as before, we have for the limiting case, $\Delta t = t_2 - t_1 = D$. Evaluating t_1 and t_2 from $I_\infty (1-e^{-\frac{t_1}{\gamma}}) = i_r$ and $\frac{I_\infty}{m}(1-e^{-\frac{t_2}{\gamma}}) = i_r$ respectively, we have

$$\Delta t = \gamma \ln \frac{I_\infty}{I_\infty - m i_r} - \gamma \ln \frac{I_\infty}{I_\infty - i_r} = \gamma \ln \frac{I_\infty - i_r}{I_\infty - m i_r} = D, \tag{4}$$

which gives

$$\frac{I_\infty}{i_r} = \frac{m e^{\frac{D}{\gamma}} - 1}{e^{\frac{D}{\gamma}} - 1} = m + \frac{m-1}{e^{\frac{D}{\gamma}} - 1}$$

Introducing $1/(e^{\frac{D}{\gamma}} - 1) \fallingdotseq \frac{\gamma}{D}$ [6] and eq. (5) (Part I), we have

$$\frac{I_\infty}{I_r} = \frac{1}{\eta}\left(1 + \frac{m-1}{mD}\gamma\right) \tag{5}$$

which shows a linear relationship between $\frac{I_\infty}{I_r}$ and γ, the most firmly established acquisition in this field[18)-21)]. In a standard case, $m \fallingdotseq 1.02$, $D \fallingdotseq 1$ msec and $\eta \fallingdotseq 1$, so the slope is about 0.02 (γ in msec), but it will change sensitively with change of m, just as β_r does.

2. Consideration

1) The linearity. This can be derived from Hill's theory, too. The differences are: a) a slight downward concavity in Hill, while a slight upward concavity in the present theory, in the range of very small γ, and b) the slope is expressed by $\frac{1}{\lambda}$ in Hill, while by $\frac{m-1}{\eta Dm}$ in the present theory.

2) "Climalyse" of Lapicque[22]. If we put $I_\infty=I_r$ in eq. (5) for the case of $\gamma=0$, then we have

$$\frac{I_r}{I_r}(=1)=\frac{1}{\eta},\ \eta>1,$$

which is evidently unreasonable. However, eq. (5) was derived from the condition $\Delta t=t_2-t_1=D$, therefore, it does not involve the case of $\gamma=0$ where $t_2-t_1=0$. We should equate

$$\frac{I_r}{I_r}=1=\frac{1}{\eta}(1+\frac{m-1}{mD}\gamma),$$

which gives

$$\gamma=\frac{Dm(\eta-1)}{m-1}$$

$(\eta-1)/(m-1)$ is of the order of 10^{-1} normally, so $\gamma\fallingdotseq\frac{Dm}{10}$, that is, γ is of the order of 10^{-1} msec, in normal frog's nerve. This γ, representing the upper limit of available time constant for starting an impulse when I_r is made to grow exponentially, is nothing but the "soeul de climalyse" termed by Lapicque for the phenomenon already established by Gildemeister[23]. If I_r is the rheobase for excitation, it is extremely difficult to understand that it can be converted into an exponentially rising one without losing its effectiveness. But we see now that it has a reason in view of the present theory.

III. Stimulation with Alternating Current

1. Intensity minimum or frequency optimum

The currents at x=0 and x=L are expressed respectively by $I_0 \sin 2n\pi t$ and $\frac{I_0}{m}\sin 2n\pi t$, I_0 being the amplitude at x=0, n frequency/sec and t time in sec. From $I_0 \sin 2n\pi t_1=i_r$ and $\frac{I_0}{m}\sin 2n\pi t_2=i_r$, we have as the condition for propagation in the limiting case,

$$\Delta t=t_2-t_1=\frac{1}{2n\pi}(\arcsin\frac{mi_r}{I_0}-\arcsin\frac{i_r}{I_0})=D$$

or

$$n=\frac{1}{2\pi D}(\arcsin\frac{mi_r}{I_0}-\arcsin\frac{i_r}{I_0})$$

Hence

$$2\pi D\frac{dn}{dI_0}=\frac{i_r}{I_0}\left(\frac{1}{\sqrt{I_0^2-i_r^2}}-\frac{m}{\sqrt{I_0^2-(mi_r)^2}}\right)<0 \tag{6}$$

and $$n_p=\frac{1}{2\pi D}\left(\arcsin\frac{1}{\eta}-\arcsin\frac{1}{\eta m}\right)\fallingdotseq\frac{1}{2\pi D}\left(\frac{\pi}{2}-\arcsin\frac{1}{m}\right), \tag{7}$$

n_p, the value of n for $I_0=I_r=m\eta i_r$

The n-I_0-relation is therefore represented schematically by the curve I in Fig. 4, which is in qualitative agreement with Hill's[15] and Tasaki's[9] theories and Hill-Katz-Solandt's[24] and Sato's[25] observations.

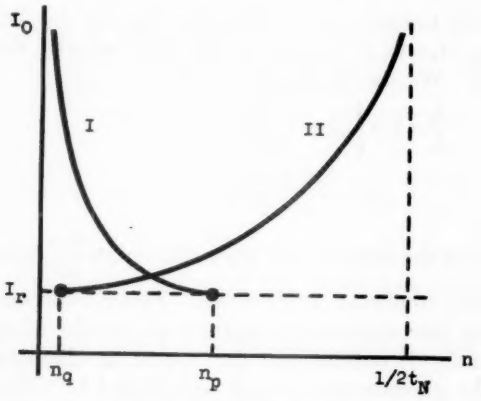

Fig. 4. $n-I_0-$relation in stimulation with alternating current. Explanation in text.

Now, there is another important condition which must be fulfilled in this case, that is $AB\geqq t_N$ (Fig. 5), where t_N is the time during which the current has to remain above the level of i_r for eliciting an excitation. Considering the limiting case $AB=t_N$,

$$AB=2\left(\frac{1}{4n}-t_2\right)=2\left(\frac{1}{4n}-\frac{1}{2n\pi}\arcsin\frac{mi_r}{I_0}\right)=t_N$$

Hence, $$n=\frac{1}{t_N}\left(\frac{1}{2}-\frac{1}{\pi}\arcsin\frac{mi_r}{I_0}\right)$$

$$=\frac{1}{2t_N}\quad\text{for } I_0=\infty,$$

$$\fallingdotseq\frac{1}{t_r}\left(\frac{1}{2}-\frac{1}{\pi}\arcsin\frac{1}{\eta}\right)\quad\text{for } I_0=I_r=m\eta i_r \text{ (n_q in Fig. 4),}$$

and $$\frac{dn}{dI_0}=\frac{1}{t_N}\frac{1}{\pi}\frac{mi_r}{I_0\sqrt{I_0^2-(mi_r)^2}}>0 \quad (t_N \text{ const., assumed}). \tag{8}$$

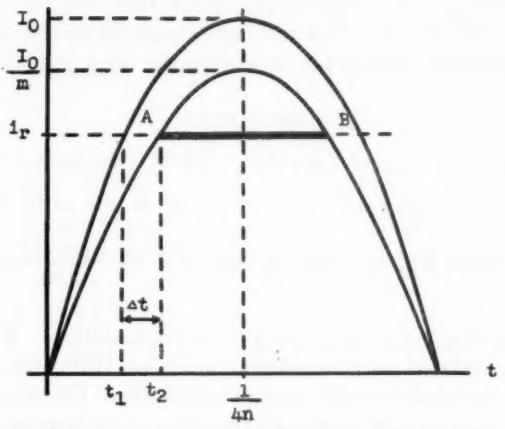

Fig. 5. Δt and t_N in stimulation with alternating current. Explanation in text.

In actuality, t_N will get smaller in stronger I_0, and, correspondingly, both $\frac{dn}{dI_0}$ and n will be larger than when t_N is constant. Further, it appears almost certain that the absolute value of dn/dI_0 is larger in eq. (8) than in eq. (6), because t_N is comparable with or even smaller than 2D in large I_0. So, the n-I_0-relation conditioned by $AB=t_N$ may be roughly illustrated by a curve like Fig. 4, II, which agrees qualitatively with Hill[15], Hill-Katz-Solandt[24] and Tasaki-Sato[26].

It is evident now that the curves I and II combined produce an I_0-minimum or n-optimum[15),24)] in the neighbourhood of n_p and a little above the level of I_r. The n-optimum actually observed is said to be about 100, while n_p evaluated from eq. (7) under $D \fallingdotseq 1$ msec is about 30, 70 and 100 for $m=1.02$, 1.1 and 1.2 respectively, that is, not much far from the observed value.

2. High thresholds in low and high frequencies

As clarified above, the I_0-n-relation is governed by the condition $\Delta t \leqq D$ in low frequencies and by $AB \geqq t_N$ in high frequencies respectively, except in a narrow range of intermediate frequencies where it is governed by both. This is easily comprehensive if we consider that, in low frequencies, both Δt and AB get lengthened, so that the condition for excitation is fulfilled but that of initiation of impulse not. The situation is just the opposite in high frequencies, that is, Δt and AB both shortened. For initiation of impulse, therefore, Δt must be reduced and AB lengthened in

low and high frequencies respectively, which both can be realized by intensifying I_0. We see thus that the high threshold in the one region has a meaning essentially different from that in the other region.

Discussion

1) Identity of β_r and I_∞-γ-line. These two lines are identical in Hill, both slopes being expressed by $\frac{I_r}{\lambda}$. It is the same in the present theory, too, except that the slope is expressed by $\frac{I_r(m-1)}{\gamma Dm}$ (compare eq. (1) and (5)).

2) β_r and I_∞-γ-line in relation to accommodation. These two lines are intimately related with accommodation in Hill's theory, while independent of threshold in the present argument. Of course we cannot deny the rise of i_r in slowly rising current without confirming it on the local excitation itself. But at all events, it is important that the lines could be shown to appear without threshold rise. As to further analysis, see Part III.

3) Mode of action of slowly rising currents as stilmuli for initiation of impulse. 1) As compared with constant current. In constant current stimulation, currents of different intensities (I_0 and $\frac{I_0}{m}$) are applied simultaneously to points $x=0$ and $x=L$. Whether an impulse is fired off or not depends upon $\Delta t \leqq D$, Δt being about the difference of the two utilization times. In stimulation with slowly rising currents, currents of one and the same intensity (i_r) are applied to the two points at two different moments ($t_{1,2}$). Whether an impulse is started or not, depends mainly upon $\Delta t = t_2 - t_1 \leqq D$. This is only a hypothesis, but it is interesting that the present treatment based on this consideration led us to good understanding of experimental facts. 2) The threshold gradient. In stimulation with linearly rising current, β_r appears as the threshold gradient. It is the same in stimulation with exponentially increasing current: Taking eq. (5) into account, we can express the stimulating current I_0 at $x=0$ generally by

$$I_0 = I_\infty (1-e^{-\frac{t}{\gamma}}) = \frac{I_r}{\eta}(1+\frac{m-1}{mD}\gamma)(1-e^{-\frac{t}{\gamma}}),\ \gamma>0$$

Hence, $$\frac{dI_L}{dt} = \frac{d}{dt}(\frac{I_0}{m}) = i_r(1+\frac{m-1}{mD}\gamma)\frac{1}{\gamma}e^{-\frac{t}{\gamma}}$$

At the moment when I_L attains i_r,

$$e^{-\frac{t}{\gamma}} = 1-1/1+\frac{m-1}{mD}\gamma = \frac{m-1}{mD}\gamma/1+\frac{m-1}{mD}\gamma$$

Hence,

$$\left(\frac{dI_L}{dt}\right)_{I_L=i_r}=i_r\frac{m-1}{mD}=\frac{\beta_r}{m}$$

or $$m\left(\frac{dI_L}{dt}\right)_{I_L=i_r}=\left(\frac{dI_0}{dt}\right)_{I_0=mi_r=I_r/\eta}=\beta_r=\text{const.}$$

This conclusion is slightly different from Tasaki's claim, $\left(\frac{dI_0}{dt}\right)_{I_0=I_r}=\beta_r$.[16] The difference appears clearest when I_r is chosen as I_∞. This case is taken in Tasaki, as in others, identical with that of $\gamma=0$, that is, $I_0=I_r=$ constant, while as one of a small γ corresponding to Lapicque's soeul de climalyse in the present theory. As a consequence, Tasaki's claim fails in this case, but the present one not. Another difference is that the threshold gradient was related to excitation in Tasaki, while to initiation of impulse in the present theory. Rudolph's recent observation[27] that a nerve fibre fails to conduct when the rising phase of the action potential is not steep enough, is very interesting in this connexion, although not clear whether the failure was of excitation or of new initiation of impulse.

Summary

1. It was shown that the following four facts can be the case without accommodation:

a) Appearance of a liminal gradient β_r in stimulation with linearly rising current.

b) Linear relationship between final current and time constant in stimulation with exponentially increasing current.

c) Repetitive responses to linearly rising current.

d) Intensity minimum or frequency optimum in stimulation with alternating current.

2. Lapicque's "soeul de climalyse" was explained.

3. Discussions were made on: a) identity of β_r and I_∞-γ-line, b) independence of β_r and I_∞-γ-line from accommodation, c) meaning of β_r, and d) modes of action of slowly rising currents as stimuli for initiation of impulse.

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* It may sound strange to consider " propagation " in experiments on single nodes. But according to my estimation[6),4a)], a certain L (0.05–0.5 μ) probably exists in each node. Therefore, "evoking a full-sized spike in a node " is equivalent to "exciting L ", that is, to "starting an impulse." A fractional excitation of a node will be possible and probable in usual methods of stimulation, because the current intensity cannot be uniform strictly.

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Excitation and Initiation of Impulse

Part III. Accommodation

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As stated in Parts I and II, distinction of excitation from initiation of impulse is very useful for understanding several phenomena of excitation, particularly some phenomena of " accommodation ", without introducing the very concept. This does never mean, however, that accommodation does not occur. As a matter of fact, some rise of I_r has been the case near[1)-3)] β_r. The point is whether the observed rise of I_r was due to rise of i_r or of m, because $I_r=m\eta i_r$ and $\eta=1+\frac{m-1}{m}\frac{1}{\frac{D}{e^{\alpha}-1}}$ (eq. (5), Part I). We will examine the matter in various cases, below.

I. Normal β_r and $\frac{I_\infty}{I_r}$-γ-line

1. In relation to i_r-rise, supposed

1) On β_r. If i_r really rises near β_r, then it will be reasonable to suppose that it will do so in larger β, too, even if little in magnitude. Let us consider therefore $i_r(\beta)$, the rheobase as a function of β, and put

$$i_r(\beta)=i_r f(\beta),$$

i_r: the value of $i_r(\beta)$ for $\beta=\infty$ (rectangular pulse),

$f(\beta)$: a function of β, which is practically unity in large β, then grows gradually with decrease of β.

Now, the gradient is β at $x=0$ and $\frac{\beta}{m}$ at $x=L$, hence $i_r(\beta)$ should be $i_r f(\beta)$ and $i_r f(\frac{\beta}{m})$ respectively. However, m is very near unity in normal conditions, so we may disregard this difference and consider just the difference due to variation of β itself.

Assuming m, D and η constant, and neglecting ε (see Part I) as before, we have for the limiting case

$$\Delta t=t_2-t_1=\frac{mi_r f(\beta_r)}{\beta_r}-\frac{i_r f(\beta_r)}{\beta_r}=\frac{i_r f(\beta_r)}{\beta_r}(m-1)=D$$

Hence, $\beta_r=\frac{i_r f(\beta_r)(m-1)}{D}=\frac{I_r}{\gamma D}\frac{m-1}{m}f(\beta_r)=$eq. (1) (Part II)$\times f(\beta_r)$ (1)

In other words, β_r gets $f(\beta_r)$-times larger than when the rhobase is invariable.

Now, eq. (1) has a meaning only when $f(\beta_r)$ is finite. Actually, I_r at β_r was found finite (1.2—1.3 times normal) as stated in Part II. This means that $f(\beta_r)=1.2-1.3$, accordingly, that β_r expressed by eq. (1) can be about as large as the standard value (see eq. (2), Part II). However, the finiteness of I_r at β_r, as a fact as well as a theoretical claim, contradicts strongly to the classical idea, which predicts ∞. Another serious point that a slight reduction of β makes the current suddenly ineffective, was described in Part II. A discontinuous change of the rheobase, from 1.2—1.3 normal to ∞, might not be impossible, but more probably there is something else still undiscovered there. This is nothing but the condition $\Delta t \leqq D$, if viewed from the present theory.

2) On $\frac{I_\infty}{I_r}$-γ-line. We take here the rheobase as a function of the rate of increase of the current. The rate is determined by I_∞ and γ, and also by time. For convenience, we will take the initial slope δ $(=\frac{I_\infty}{\gamma})$ and put $i_r(\delta)=i_r F(\delta)$,

i_r: the value of $i_r(\delta)$ for $\delta=\infty$ (rectangular pulse),

$F(\delta)=F(\frac{I_\infty}{\gamma})$: a function similar to $f(\beta)$.

For the points $x=0$ and $x=L$, we should put, strictly,

$$I_\infty(1-e^{-\frac{t_1}{\gamma}})=i_r F(\delta),$$

and $$\frac{I_\infty}{m}(1-e^{-\frac{t_2}{\gamma}})=i_r F(\frac{\delta}{m}).$$

But if taken $F(\delta)\fallingdotseq F(\frac{\delta}{m})$ as in the case of linearly rising current, then

$$t_1=\gamma \ln\frac{I_\infty}{I_\infty-i_r F(\delta)} \text{ and } t_2=\gamma \ln\frac{I_\infty}{I_\infty-mi_r F(\delta)}$$

In the limiting case

$$\Delta t=t_2-t_1=\gamma \ln\frac{I_\infty-i_r F(\delta)}{I_\infty-mi_r F(\delta)}=D$$

The solution is

$$\frac{I_\infty}{i_r}(=\frac{I_\infty \gamma m}{I_r})=F(\delta)\frac{me^{-\frac{D}{\gamma}}-1}{e^{\frac{D}{\gamma}}-1}=F(\delta)(m+\frac{m-1}{e^{\frac{D}{\gamma}}-1})$$

$$\fallingdotseq F(\delta)\{m+(m-1)\frac{\gamma}{D}\} \quad \left(\frac{1}{e^{\frac{D}{\gamma}}-1}\fallingdotseq\frac{\gamma}{D} \text{ introduced}\right)$$

$$\therefore \quad \frac{I_\infty}{I_r}=\frac{F(\delta)}{\eta}(1+\frac{m-1}{m}\frac{\gamma}{D}) \qquad (2)$$

By assumption, $F(\delta)$ is a function which, growing from unity, gets larger with decrease of δ. We cannot foretell precisely how δ will change with γ, because we do not know how I_∞ will change with γ, this being the very thing which we wish to clarify. But it is certain that δ starts with a very large value, and, decreasing with increase of γ, reaches a certain value, δ_r, which corresponds to β_r. $i_r(\delta)$ will then be $i_r F(\delta_r)$. If taken $F(\delta_r)=f(\beta_r)=1.2-1.3$, then eq. (2) may be said to be practically linear, although never so strictly except in the range of large δ where $F(\delta)\fallingdotseq 1$.

2. In relation to *m*-rise, supposed

A slowly rising cathodic current will probably loosen the membrane and thus diminish the resistance r_m (see Suzuki[4]). This makes k smaller, which makes L larger in turn (see Fig. 1, Part I). A further enlargement of L is expected from reduction of f due to diminution of V amounting to 50% of the normal at β_r[1]. There are also factors which act to diminish L, namely,[1] enlargement of f by decrease of r_m[5], and 2) some possible decrease of k', although presumably very little. Even if L remains unchanged after all these changes, still m can augment as the result of k-decrease alone. The rise of m seems thus highly probable.

1) On β_r. Assume i_r, D, α and practically also η constant, and further

$$m(\beta)=mf(\beta),$$

m: the value of $m(\beta)$ for $\beta=\infty$ (rectangular pulse),
$f(\beta)$: the same function as before.

From $I_0=\beta t_1=i_r$ and $I_L=\frac{\beta}{mf(\beta)}t_2=i_r$, and under neglect of ε as before, we have for the limiting case

$$\Delta t=t_2-t_1=\frac{i_r}{\beta_r}\{mf(\beta_r)-1\}=D;$$

$$\beta_r=\frac{i_r}{D}\{mf(\beta_r)-1\}=\frac{I_r}{\eta D}\frac{mf(\beta_r)-1}{m}$$

$$\text{or} \quad =\frac{I_r}{\eta D}\frac{m-1}{m}\frac{mf(\beta_r)-1}{m-1}=\text{eq. (1) (Part II)}\times\frac{mf(\beta_r)-1}{m-1} \qquad (3)$$

In a standard case, eq. (1) (Part II) turns into eq. (2) (Part II), which gives $\beta_{r\cdot o}=0.6$ mV/msec. Hence, if taken $f(\beta_r)=1.2-1.3$ in eq. (3) above, then $\beta_r\fallingdotseq 0.6\times(11-16)\fallingdotseq 7-10$ mV/msec, which is larger than the largest value obtained by Diecke[1]. This suggests that rise of m in linearly

rising current is improbable, as long as $f(\beta_r)$ is so large as 1.2—1.3.

2) On $\frac{I_\infty}{I_r}$-γ-line. Suppose that $m(\delta)=mF(\delta)$, and i_r, D and η constant.

From $I_\infty(1-e^{-\frac{t_1}{\gamma}})=i_r$ and $\frac{I_\infty}{mF(\delta)}(1-e^{-\frac{t_2}{\gamma}})=i_r$, we have

$$t_1=\gamma \ln\frac{I_\infty}{I_\infty-i_r} \text{ and } t_2=\gamma \ln\frac{I_\infty}{I_\infty-i_r mF(\delta)};$$

$$\Delta t=\gamma \ln\frac{I_\infty-i_r}{I_\infty-i_r mF(\delta)}=D;$$

$$\frac{I_\infty}{i_r}=\frac{mF(\delta)e^{\frac{D}{\gamma}}-1}{e^{\frac{D}{\gamma}}-1}=mF(\delta)+\frac{mF(\delta)-1}{e^{\frac{D}{\gamma}}-1}$$

Introducing $\eta mi_r=I_r$ and $\frac{1}{e^{\frac{D}{\gamma}}-1}\fallingdotseq\frac{\gamma}{D}$,

$$\frac{I_\infty}{I_r}=\frac{F(\delta)}{\eta}\left(1+\frac{m-\frac{1}{F(\delta)}}{m}\frac{\gamma}{D}\right) \qquad (4)$$

Eq. (4), like (2), can be linear in the range of $F(\delta)\fallingdotseq 1$ only. The final slope is larger than that of eq. (2), so the linearity is the worse as a whole.

3. Consideration

Two important conclusions are drawn from the above examinations:

1) A rise of I_r, whether due to rise of i_r or m, not only contradicts to the appearance of β_r of a reasonable value, but also disturbs the linearity of I_∞-γ-curve, except when the rise is very small. At least, it never favours the appearance of these lines, as was foreseen already in Part I.

2) The rise of I_r near β_r appears to be due to rise of i_r, not of m. This is, however, because $f(\beta_r)$ is so large as 1.2—1.3. If it is smaller, then the matter turns out quite different. The possibility will be stated in Discussion below, but before that we will examine the matter in some other cases.

II. I_r and β_r in Electrotonus

It is known that I_r and β_r both rise in long-lasting C.E.T. (Tasaki[6]). A remarkable thing thereby is that the rise of I_r is just little while that of β_r enormous. In Tasaki's observation above quoted, " the rheobase was not appreciably changed " but β_r changed from $\frac{1}{62}$- to $\frac{1}{18}$-msec^{-1}, that is, to about 3.5-times as large.

If wanted to understand this unproportionality from Hill's theory, we have to consider two events, a small rise of the rheobase and a large reduction of λ, taking place simultaneously. But if viewed from the present theory, it is comprehensive as a simple result of m-rise only:

Eq. (5) (Part I): $I_r = m\eta i_r$

Eq. (1) (Part III): $\beta_r = \frac{i_r(m-1)}{D} f(\beta_r)$ (i_r varying with β)

Eq. (3) (Part III): $\beta_r = \frac{i_r}{D}\{m f(\beta_r) - 1\}$ (m varying with β).

If C.E.T. raises i_r, then both I_r and β_r should rise in the same proportion, but if it raises m, then I_r rises just proportionally as before, while β_r very much more. Quantitatively, eq. (1) (Part III) is satisfactory, because a rise of m by just 5% (from 1.02 to 1.07) suffices to explain Tasaki's result. Eq. (3) (Part III) is not available, again because $f(\beta_r)$ is so large as 1.2—1.3.

If the above consideration is correct, then in A.E.T. a little decrease of m (from 1.02 to 1.00 at most), accordingly of I_r, and a relatively large diminution of β_r are expected. In actuality, β_r decreased to $\frac{1}{900}$ msec^{-1}, but I_r increased from 25 to 100 mV in Tasaki's experiment above quoted. The reason of this discrepancy is not clear, but one might be that in Tasaki the stimulus was expressed in voltage, which could have acted only through the membrane resistance, which must have been increased in A.E.T.

III. I_r and β_r in Low Temperature

As quoted in Part II, I_r changes very little but β_r gets very small in low temperature (Tasaki[7]). We have here another example of unproportional change of I_r and β_r. It must be due partly to prolongation of D as stated before, but very probably also to diminution of m, which, if occurred in a standard case, cannot be large only from 1.02 to 1.0 at most), but still can bring forth an enormous decrease of β_r.

IV. I_r and β_r in Relation to Na^+-concentration

It may not be appropriate to discuss the complicated effects of ions upon excitability, on the same line with that of electric current. Here just one example which is very interesting in connextion with the present theory, will be quoted.

According to Diecke[1], both I_r and β_r decrease with increase of Na^+-concentration of the bathing fluid. Here again, the decrease of β_r is much more marked than that of I_r (Abb. 11). For example, at $[Na^+]=444$ mM/l, β_r was already so small as 44%, while I_r remained at about 80%, of the

normal (see Tab. 1 and Abb. 11). This unproportional change of I_r and β_r seems to point to m-change taking place in this case, too. In Diecke's preparations, the value of m is seemingly near about 1.2 as stated in Part II. If m ran down to 1.1 in the above example, I_r must have decreased by about 10%, while β_r by just 50% (eq. (1), Part II), which is about in accordance with the actual observation. Why m decreases in higher Na-concentrations, is not clear, but decrease of L due to increase of V, accordingly of f, the safety factor, must be a powerful agent.

V. Repetitive Responses

1. To constant current

The limiting condition for initiation of impulse is

$$\text{Eq. (4) (Part I)}: \quad \Delta t = \alpha \ln \frac{I_0 - i_r}{I_0 - m i_r} = D$$

In Part I, the characteristics of the repetitive responses were interpreted as due to gradual rise of α with time. However, I_0 being long-lasting, there are two other possibilities, namely, rise of i_r and m. If they increase, they exert just similar effects as α-increase upon t_2 and Δt, so that the known features of repetitive responses can be explained similarly. Here, just some consideration will be carried out on the time-length, during which the response is repeated.

1) Rise of i_r with time and I_0, supposed, under m, D, α constant. Replacing i_r with $i_r(I_0, t)$ in eq. (4) (Part I), we have

$$I_0 - i_r(I_0, t) = e^{\frac{D}{\alpha}} (I_0 - m\, i_r(I_0, t)),$$

from which $i_r(I_0, t) = I_0 \dfrac{e^{\frac{D}{\alpha}} - 1}{m e^{\frac{D}{\alpha}} - 1} = I_0 \dfrac{1}{\eta m}$ (see eq. (5), Part I) This is the relation between i_r (raised) and I_0 (applied) when the response is just stopping.

Put now $i_r(I_0, t) = i_r f(I_0, t)$, where i_r is the value of $i_r(I_0, t)$ at $t=o$ and $f(I_0, t)$ a function growing from unity, and introduce it into the above equation. Then

$$f(I_0, t) = \frac{I_0}{i_r \eta m} = \frac{I_0}{I_r} \tag{5}$$

2) Rise of m with time and I_0, supposed, under other variables unchanged

Replacing m in eq. (4) (Part I) with $m(I_0, t)$, we have

$$m(I_0, t)\; i_r\, e^{\frac{D}{\alpha}} = I_0 e^{\frac{D}{\alpha}} - I_0 + i_r$$

$$m(I_0, t) = \frac{I_0}{i_r} (1 - e^{-\frac{D}{\alpha}}) + e^{-\frac{D}{\alpha}} = \frac{I_0}{I_r} m\eta (1 - e^{-\frac{D}{\alpha}}) + e^{-\frac{D}{\alpha}}$$

Putting $m(I_0, t)=mf(I_0, t)$, m being the value of $m(I_0, t)$ at $t=0$,

$$f(I_0, t)=\frac{I_0\eta}{I_r}(1-e^{-\frac{D}{a}})+\frac{1}{m}e^{\frac{D}{a}} \qquad (6)$$

The values of t which satisfy eq. (5) or (6) respectively determine the "total adaptation time, T" in Granit and Skoglund's terminology[8]. Presumably, eq. (6) will fit the observations better, because the case under examination is a special case of C.E.T., where the rise of m seems certain, as stated before. Unfortunately, the form of $f(I_0, t)$ is unknown, so we cannot formulate T as a function of I_0, but it is interesting to make a simple assumption that $f(I_0, t)=f(t)$, that is, that i_r or m is a function of t only, and to consider two special cases, 1) $f(t)=e^{\frac{t}{p}}$, p constant, and 2) $f(t)=\ln(e+\frac{t}{q})\fallingdotseq\ln\frac{t}{q}$ in large $\frac{t}{q}$. The former, combined with eq. (5), leads to $t=p\ln\frac{I_0}{I_r}$. If p is replaced by λ, then we have just the equation derived by Katz[9] on the basis of Hill's theory. Katz's equation represents therefore a special case where $f(t)=e^{\frac{t}{\lambda}}$ is introduced, which, in view of the present theory, has no proper reason. It is not strange that it does not fit the observations well[8]. On the other hand, the latter combined with eq. (6) leads to

$$\ln t=\frac{I_0}{I_r}\eta(1-e^{-\frac{D}{a}})+\frac{1}{m}e^{-\frac{D}{a}}+\ln q=c_1\frac{I_0}{I_r}+c_2$$

$$(c_1=\eta(1-e^{-\frac{D}{a}});\ c_2=\frac{1}{m}e^{-\frac{D}{a}}+\ln q),$$

a linear relationship between $\ln t$ and $\frac{I_0}{I_r}$ as long as $\frac{D}{a}$ remains unchanged. This supports Granit and Skoglund[8] who demonstrated a practical linearity of the $\ln T$-$\frac{I_0}{I_r}$-curve, and suggests, conversely, a logarithmic rise of m with t strongly.

2. To linearly rising current

Eq. (3) (Part II) was derived from eq. (4) (Part I), regarding i_r and m constant. Here we assume them to be a function of β and T.

1) $i_r(\beta, T)=i_r f(\beta)F(T)$, supposed

i_r: the value of i_r (β, T) at $\beta=\infty$ and $T=0$,

$f(\beta)$: the same function as before,

$F(T)$: a function of T growing from unity; the end of t_0 taken as origin of T (Fig. 2, Part II)

Assume m=constant, and consider the case of $\beta=\beta_r$. Then, in the same way as in Part II,

$$\Delta t_n=\alpha \ln\frac{i_r+\beta_r T_{n-1}-i_r f(\beta_r)F(T_n)}{i_r+\beta_r T_{n-1}-i_r mf(\beta_r)F(T_n)}$$

$$=\alpha \ln\frac{1-\dfrac{i_r f(\beta_r)F(T_n)}{\beta_r T_{n-1}}}{1-\dfrac{i_r mf(\beta_r)F(T_n)}{\beta_r T_{n-1}}} \quad (i_r \text{ neglected against } i_r f(\beta_r)F(T_n))$$

$$=\alpha \ln\frac{1-\dfrac{D}{m-1}\dfrac{F(T_n)}{T_{n-1}}}{1-\dfrac{mD}{m-1}\dfrac{F(T_n)}{T_{n-1}}} \quad (\text{eq. (1), Part III, introduced})$$

$$\fallingdotseq\alpha \ln\frac{1-DM\dfrac{F(T_n)}{T_n}}{1-DMm\dfrac{F(T_n)}{T_n}} \left(M=\frac{1}{m-1}\ ;\ T_{n-1}\fallingdotseq T_n, \text{ assumed}\right)$$

2) $m(\beta,\ T)=mf(\beta_r)F(T)$, supposed

$$\Delta t_n=\alpha \ln\frac{i_r+\beta_r T_{n-1}-i_r}{i_r+\beta_r T_{n-1}-i_r mf(\beta_r)F(T_n)}$$

$$=\alpha \ln\frac{\beta_r T_{n-1}}{\beta_r T_{n-1}-i_r mf(\beta_r)F(T_n)} \quad (i_r \text{ neglected against } i_r m f(\beta_r)F(T_n))$$

$$\fallingdotseq\alpha \ln\frac{1}{1-D\dfrac{mf(\beta_r)}{mf(\beta_r)-1}\dfrac{F(T_n)}{T_n}} \quad (\text{eq. (3), Part III, introduced, and } T_{n-1}\fallingdotseq T_n, \text{ assumed})$$

$$\fallingdotseq\alpha \ln\frac{1}{1-DN\dfrac{F(T_n)}{T_n}} \left(N=\frac{mf(\beta_r)}{mf(\beta_r)-1}\right)$$

The quantity $\dfrac{F(T_n)}{T_n}$ represents the mean rate of i_r- or m-rise. If this gets larger with T, then Δt doing so too, the repetitive response will cease after a certain while. But if it decreases sufficiently rapidly or if, in addition, a larger β is chosen, then the response will continue on without cessation. The practical necessary condition for this is that the rate of rise of i_r or m gets smaller with time. The logarithmic nature of m-rise suggested above, will probably satisfy this condition, to establish Hoffmann's observation[10], provided that α-rise is relatively little.

Discussion

1. β_r and I_∞-γ-line, and their probable independence of I_r-rise.

In Part II, it was shown that β_r and I_∞-γ-line can be the case without threshold rise; in Part III that threshold-rise, if it occurs, disturbs the appearance of β_r of a reasonable value, and of the linear relationship between I_∞ and γ. These results, together with the fact that I_r is certainly finite at β_r in contradiction to the theory of accommodation, point to independence

of the threshold from β_r and I_∞-γ-line.

2. I_r-variation and its probable dependence on m-rise.

It is sure that I_r varies according to conditions. But as far as examined above, it seems to be m that changes in electrotonus, temperature varied, in varied Na^+-concentration of the bathing fluid, and also in repetitive responses to constant and linearly rising currents. The only exception encountered is the I_r-rise observed near β_r, where the rise of i_r appeared responsible. One possibility might be that i_r changes in short-lasting while m changes in long-lasting exposure to new conditions. There are, however, also reasons to believe that the actual rise of I_r near β_r might have been much smaller than observed: 1) the time of ascent (up to the I_r-level) is only several ten msec in most cases, which seems to be too short to exhibit any appreciable depressive action, 2) in Sugi's experiment[11] with linearly rising current, the range of constant threshold was so wide that even β_r could hardly be confirmed, and 3) in stimulation with exponentially increasing current, the excitation always took place at an almost constant level of potential[1),3)]. All these facts point to threshold constancy in stimulation with slowly rising currents. I dare suppose: was the I_r-rise near β_r not an artifact, at least in part, due to polarization still remaining on isolated fibres? Was the actual rise not much smaller and this small rise not due to rise of m? This might be a wilful supposition, but if it is correct, and if we can put $f(\beta_r) \fallingdotseq 1$, then eq. (1) and (3) in Part III are both reduced to eq. (1) or (2) in Part II which provide a reasonable value of β_r; and eq. (2) and (4) in Part III to eq. (5) in Part II, which expresses a strict linearity of $\frac{I_\infty}{I_r}$-γ-curve. A unific explanation can thus be presented by rise of m. Further, the rise of m, thus supposed, needs be just small. If it is large, it makes β_r too large, $\frac{I_\infty}{I_r}$-γ-curve not linear, the total adaptation time for constant current too short, and the ever-lasting repetitive responses to linearly rising current with $\beta > \beta_r$ impossible. It is quite interesting in this connexion that just a 5% increase of m was found enough for explaining the facts in C.E.T., as stated above.

3. "Accommodation" in terms of I_r- or m-rise

"Accommodation" is certain as long as a rise of I_r is meant by it. In view of the present theory, however, I_r is different from i_r, and the rise of I_r is highly probably due to rise of m. The so-called accommodation becomes then a matter of initiation of impulse having nothing to do with i_r, the rheobase for excitation. I dare propose therefore to define accommodation practically by rise in I_r, or theoretically by rise in m, and to call m the "constant of propagation" for convenience.

The point is whether the distinction of excitation from initiation of impulse is correct or not. However, the identification of these two has no basis, either. If the distinction has any reason, and the accommodation is really a matter concerning initiation of impulse, then all the explanations for the mechanism of accommodation on the basis of i_r-rise, including the modern one of Hodgkin[12], though to be questioned or reconsidered.

Summary and Conclusions

1. β_r of the observed value and the linear relationship between $\frac{I_\infty}{I_r}$ and γ cannot be expected from the present theory, unless the rise of I_r is very small.

2. β_r and $\frac{I_\infty}{I_r}$-γ-line are very probably independent of I_r-rise.

3. Variation of m supposed to occur to a small extent, was shown to be very useful for explaining various experimental facts. The rise of I_r near β_r was supposed to be due partly to artifact, and partly to rise of m.

4. It was proposed to define accommodation by rise in I_r or m, and to call m the constant of propagation.

5. Discussions were made on 1) probable independence of β_r and $\frac{I_\infty}{I_r}$-γ-line from I_r-rise, 2) probable dependence of I_r on m, and 3) accommodation.

6. General conclusions from Part I, II and III.

1) Distinction between excitation and initiation of impulse is very useful for explaining experienced facts.

2) The rise of m, not of i_r, to a small extent seems to be a general basic event that happens in normal nerves in various conditions.

3) Of the well-known three factors of stimulating currents, a) intensity ($>i_r$), b) duration ($>$utilization time) and c) slope ($>\beta_r$), the third one appears to be an additional requisite for initiation of impulse.

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Retinal Processes in Total Color Blindness

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Introduction

In many cases of total color blindness[1)-4)] the course of dark adaptation was shown to consist of two parts in a manner very similar to the normal dark-adaptation curve. As the earlier and the later parts of the normal curve are usually interpreted as representing cone-thresholds and rod-thresholds respectively, the question has arisen as to whether or not the two portions of the curve obtained from a total color-blind might be accounted for in terms of cone- and rod-thresholds.

Based upon the day-rod theory propounded by von Kries, Sloan[3)] ascribed the earlier part of the curve obtained from an achromat to the activity of high-threshold rods. On the contrary, Walls[4)] claims that cones of a special type, that is, "B cones" would be responsible for the earlier part of the curve.

In the present experiment, we analyzed the retinal processes of two typical congenital achromats by means of the method of electrostimulation, and found that there are two kinds of retinal process concerning scotopic vision.

Experimental

Method

One of our subjects was a typical congenital achromat aged 18. Her elder sister was a total color-blind, too. In this subject a relative central scotoma was found, although its boundary was not clear. Both photophobia and nystagmus were present. The other subject was a 36 years old man. He had four brothers among whom there was a total color-blind. In him no central scotoma was found. Photophobia and nystagmus were only slight. Color vision tests were made with Nagel's anomaloscope.

Electrical measurements were carried out as follows. After preliminary dark adaptation of about 20 minutes, the electrical sensitivity of the eye was measured with a rectangular pulse of 0.1 second. Similar measure-

ments were carried out at varying times following a brief illumination lasting 0.5 seconds in most cases to determine the time course of the electrical excitability after the light stimulus. The effect of the light stimulus was expressed by a quantity ζ which represents a percentage increase in electrical excitability caused by the photic stimulus.

We were afraid that the accuracy of electrical measurements might be lowered by the presence of nystagmus, but in reality, determination of electrical thresholds could be done in both subjects with higher accuracy and greater ease than in most normal beginners. The reason is not clear.

It may be supposed that the simpler receptive mechanism in achromats would be favorable for such electrical measurements, because any confusion due to concurrent excitation of different receptive systems would not occur in them.

Results

1. *The twin process and the rod process*

Motokawa *et al.*[5] showed that the curve of electrical excitability of the normal dark-adapted eye has a maximum at 4.5 seconds from the onset of a brief light stimulus of low intensity. This process was called " the rod process ", because the height of the maximum depends on the wavelengths of spectral lights in the same manner as does the scotopic visibility.

Oikawa[6] found another kind of achromatic process which shows two maxima at 1.5 and 3.0 seconds from the end of the light stimulus, and designated the process " the twin process ". The rod process is related to on-elements of the retina, because its crest appears always at 4.5 seconds from the onset of the photic stimulus. The twin process may be correlated with pure off-elements or on/off-elements, because its crests are closely connected with the end of the photic stimulus.

When the intensity of any spectral light lies within certain limits, it elicits a sensation of light, but no sensation of hue. This range of intensities is, therefore, called the photochromatic interval for that kind of light. It is only within the photochromatic interval that the twin process appears in the normal retina. The rod process appears over a much wider range in the normal retina. Thus the two kinds of achromatic processes are different not only with respect to the physiological mechanisms involved, but also in functional ranges of intensities.

In Fig. 1, the rod process and the twin process obtained from our achromatic subjects are illustrated. It is to be noted that the only processes obtainable in achromats are such achromatic processes. The upper curve was obtained from subject A with spectral light of 510 mμ. There can be seen three maxima in the curve of which the earlier two belong to the twin process, and the latest one represents the rod process. As the

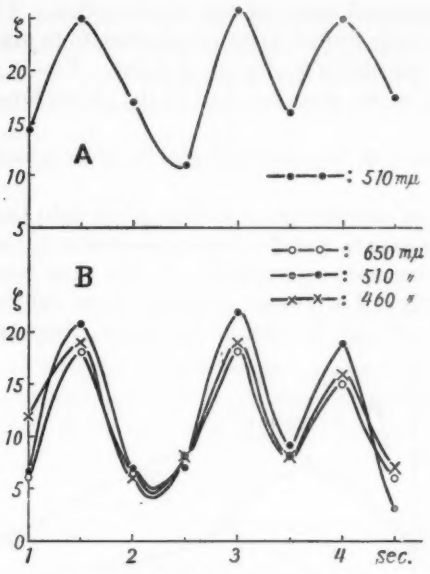

Fig. 1. ζ-time curves obtained from two achromats, A and B. Ordinates: Percentage increases in electrical excitability over resting level. Abscissae: Time after end of illumination lasting 0.5 sec. Earlier two peaks belong to twin process, and the latest one to rod process.

maximum at 1.5 seconds appears always hand in hand with the one at 3.0 seconds, they constitute a unit process. The twin process composed of two maxima at 1.5 and 3.0 sec. can be separated from the rod process in the following manner: If the duration of the light stimulus is made longer than 4 seconds, the rod process does not appear any more, probably because the process started at the onset of the stimulus comes to an end during the illumination. On the contrary, the twin process always appears how long the duration may be.

As has been shown by Motokawa and others, the retinal color processes which are caused by yellow light and by blue light have a maximum at 1.5 and 3.0 seconds respectively from the end of the light stimulus. Since the yellow process and the blue one agree with the two components of the twin process in their time course, it may be said that the twin process is composed of two complementary processes, yellow and blue, and that this constitution would be responsible for the achromatic function of the twin process.

In Fig. 1B, ζ-time curves for three different spectral lights obtained from subject B are illustrated. The time courses agree with one another

within the experimental error of our investigation. This fact indicates that the form of each retinal process obtained from the achromat is independent of the wavelengths of spectral lights. This is also the case with the normal, so far as the processes seen in the photochromatic interval are concerned.

2. *Dependence of the two achromatic processes of the achromat on the intensities of illumination*

In this series of experiments, a flash of white light lasting 40 msec. was used for pre-illumination, and ζ-values measured at 3.0 and at 4.5 seconds from the flash were used as measures for the twin process and the rod process respectively. The results obtained from subjects A and B are shown in Fig. 2. As can be seen in this figure, there are optimal ranges

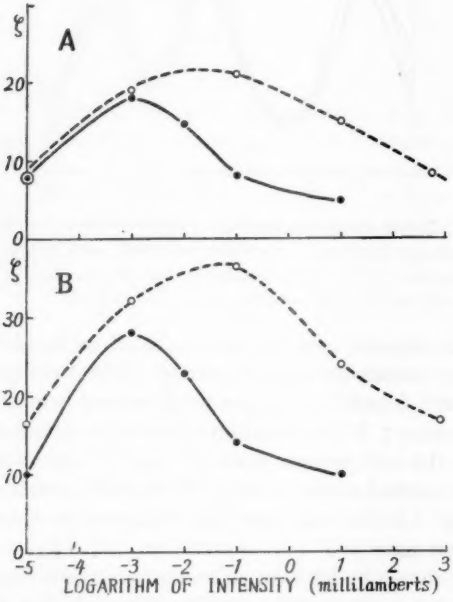

Fig. 2. Dependence of rod process (continuous curves) and of twin process (broken curves) on intensities of illumination lasting 40 msec. A and B represent results obtained from two achromats.

of intensity for the two kinds of processes; the optimal intensity for the rod process is 10^{-3} millilamberts, while that for the twin process is 10^{-1} millilamberts. It is apparent that the twin process has a much wider working range of intensities than does the rod process. It is to be noted that this relation holds only for the achromat; in the normal the working range for

the twin process is limited to the photochromatic interval, while the rod process can appear above the color threshold above which the twin process is replaced by the color process specific to the spectral light used. In any case, the two kinds of achromatic processes differ from one another with respect to their functional ranges of intensities.

3. *Spatial distribution of the two kinds of processes in the retina*

We have mentioned so far about the differences in properties of the two kinds of achromatic processes. In what follows, the properties common to both processes will be described.

The spatial distribution of each process in the retina was investigated, measuring ζ-values at various points on the temporal horizontal meridian of the retina. The values measured at 4.5 sec. from a flash of white light of a certain definite intensity were taken as a measure for the rod process and the values measured at 1.5 or 3 sec. for the twin process. The data obtained from subject B are shown in Fig. 3. The upper curve represents

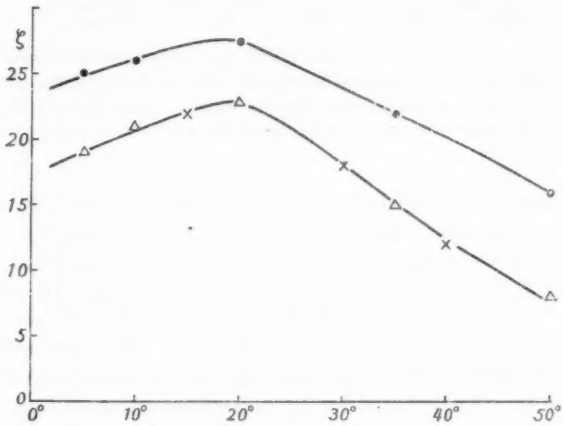

Fig. 3. Spatial distribution of rod process (curve marked by dots) and of twin process (curve marked by triangles and crosses). Abscissae : degrees of eccentricity from center of fovea.

the spatial distribution of the rod process, while the lower refers to the twin process. As can be seen in this figure, the two curves run parallel to each other, showing a maximum at about 20° from the fovea. These distributions of the two processes resemble the spatial distribution of the density of rods determined by Østerberg.

4. *The spectral distribution of the rod process and the twin process obtained from achromats*

The two kinds of achromatic process behave alike not only in the

spatial distribution in tle retina, but also in the spectral distribution. This relation is illustrated in Fig. 4. The continuous curve connecting solid

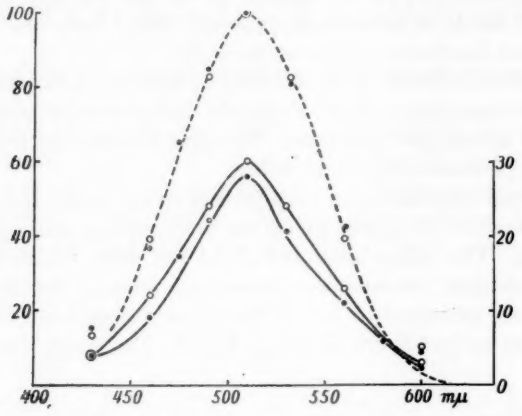

Fig. 4. Spectral distribution of rod process (continuous curve marked by open circles) and of twin process (continuous curve marked by dots). Broken curve represents scotopic visibility curve by Wright. If expressed in per cent of maximal magnitude, curves for two processes agree with scotopic visibility curve.

circles represents the magnitudes of the twin process expressed by ζ values at 3.0 sec., and the one connecting empty circles refers to the rod process whose magnitudes are expressed by ζ values measured at 4.5 sec. from the onset of the light stimulus. The spectrum used was an equal-energy spectrum. As can be seen in this figure, both curves are very similar in shape, having a maximum at about 507 mμ. The agreement of both curves is more perfect when the data are expressed in percentage of the maximal values. As is shown in the uppermost curve of Fig. 4, the data for the two kinds of process can be represented by a single curve. In other words, the two processes are identical with respect to the wavelength-dependence, and this fact may most easily be understood, if it is assumed that one and the same photochemical substance, visual purple, mediates the spectral sensitivity of both processes.

The broken curve in this figure was drawn with Wright's[8] data for scotopic visibility of normals. It is surprising how closely our curve concerning achromatic process of the achromat agrees with the scotopic visibility curve of the normal. Motokawa *et al.*[5] and Oikawa *et al.*[7] showed that the spectral distribution curve of the rod process obtained from the normal fairly coincided with the normal scotopic visibility curve, but the agreement was not so satisfactory in their experiments on normals as in

the present experiment on achromats. In Oikawa's experiment, the agreement became more and more satisfactory, as the measuring area moved further into the periphery of the retina. Oikawa accounted for this finding in terms of interaction between cones and rods. If the less satisfactory agreement found in the central parts of the retina is really due to some effect of cones upon rods, as Oikawa surmises, it is to be expected that in achromats agreement is more satisfactory, because there would be no or few cones to interfere with the activity of rods. Our experiment on achromats furnishes a verification of the notion.

5. *Dark-adaptation curves of achromats*

As has been mentioned above, dark-adaptation curves of some achromats show a kink similar to the one known as Kohlrausch's kink in the normal dark-adaptation curve. In order to see whether or not any kink appeared in our subjects, the following measurements were carried out after a preliminary light adaptation for 5 minutes to white light of 1000 millilamberts. The target used was 30 minutes or 1 degree in visual angle. It was presented just above a fixation point. Measuremts of light thresholds were done with Nagel's adaptometer.

Two dark-adaptation curves obtained from subject B are illustrated in Fig. 5. As can be seen in this figure, the curves consist of two parts,

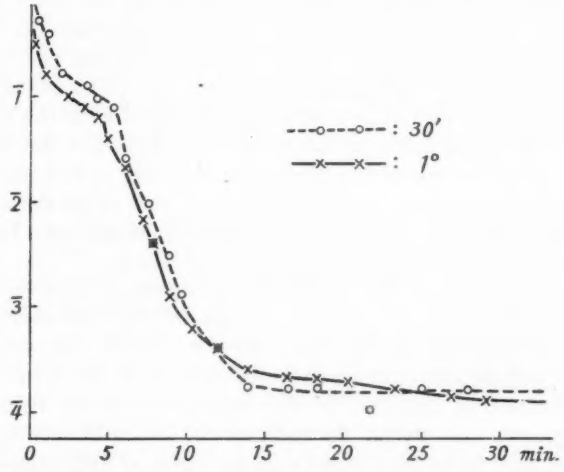

Fig. 5. Dark-adaptation curves obtained from achromat, using test patches 30′ and 1° in visual angle. Ordinates: logarithms of threshold intensities (white light). Abscissae: time in minutes after onset of dark adaptation.

showing a kink at about 5 minutes from the onset of dark adaptation.

Discussion

As has been shown above, the twin process and the rod process are two different processes distinguishable by the time course of electrical excitability following illumination, but have common properties concerning spatial distribution as well as spectral distribution. Judging from their spectral distribution, it may be said that both processes are mediated by rhodopsin. Oikawa suggested that some difference in nervous connections in the retina may be responsible for the difference between the two processes, while the photochemical substance is the same for both processes. As has been shown above, the time courses of the two components of the twin process are the same as that of the yellow process and that of the blue process respectively. From these findings it may be supposed that some nervous elements produce a color process like the yellow or blue process, when connected with certain cone receptors, but produce a twin process, when linked with rod receptors.

In any case, it is so important a fact that there are the two kinds of achromatic process in the retina of the achromat, because it furnishes an explanation for the puzzle concerning the dark-adaptation curve of the achromat mentioned above.

Some investigators[3] are of the opinion that the cone receptors subserving blue sensations participate in scotopic vision, on the basis of their experience that bluish-white sensations occur in response to achromatic stimuli under dark adaptation. Walls[4] ascribed the first part of the dark-adaptation curve of the achromat to the cone receptors of this type. As a matter of fact, no retinal process to be identified with the blue process has so far been found in the retina of the achromat, but the two kinds of achromatic processes mentioned above are the only ones found in it.

As stated above, Sloan ascribed the earlier part of the dark-adaptation curve of the achromat to hypothetical high-threshold rods, but evidence for the exsistence of the high-threshold rods is lacking.

As is shown in Fig. 2, the magnitudes of responses are of the same order for both processes, when the intensity of illumination is lower than a certain limit, 10^{-3} millilamberts in this case. In other words, the scotopic sensitivity of the rod process and the twin process is of the same order. At higher intensities of illumination the magnitude of the twin process is decidedly greater than that of the rod process, as can be seen in the figure. This finding may be so interpreted that the effect of light adaptation is far stronger upon the rod process than upon the twin process. The twin process which is more resistant to light adaptation will work predominantly over the rod process in the earlier stage of dark adaptation, but in the later stage the rod process will work togerther with the twin process. Thus the two parts of the dark-adaptation curve of the achromat will be established.

This is our explanation of the question raised by the previous investigators. We assume neither any participation of cones in achromatic vision nor existence of high-threshold rods, because no evidence for these assumptions is available. The difference in susceptibility to light adaptation of our two processes cannot be ascribed to any difference in photosensitivity of the rods concerning the two processes. Both processes are equally sensitive under dark adaptation, as has been mentioned above, and their spectral distribition of sensitivity is identical. These findings suggest that the rods initiating the two processes are equally sensitive. There is no reason to suppose that the respective rods should show different sensitivity only under light adaptation. We think the difference would be due to some difference in nervous organization for both processes.

Granit and coworkers[9] reported that following high states of light adaptation, rhodopsin began to regenerate at once, but that the b-wave of the ERG remained very small (frog) or wholly absent (cat) until the rhodopsin had reached about 40 to 50 per cent of its maximal concentration. Wald[10] advanced an elaborate hypothesis concerning this relation, but he did not take any nervous mechanism into consideration which might be responsible for the discrepancy between the photochemical and neurophysiological phenomena mentioned above.

The decrease in magnitude of the rod process at too high intensities of illumination (see Fig. 2) may be accounted for in terms of neurophysiological inhibition more easily than in terms of the photochemical reaction. The higher susceptibility of the rod process to light adaptation compared with the twin process should likewise be ascribed to greater liability to inhibition of the nervous mechanism underlying the rod process.

SUMMARY

Retinal processes in two typical achromats were investigated by the method of electrostimulation. From measurements of time courses of electrical excitability of the eye following a brief illumination, two kinds of process were distinguished.

1. The time courses of the two processes showed no dependence on wavelengths of lights, and their magnitudes varied like the scotopic visibility curve, as the wavelengths of spectral lights were varied. Therefore they were interpreted as subserving achromatic vision.

2. The spatial distribution of both processes was found very similar to that of the density of rods in the retina.

3. The two processes differed, however, with respect to intensity ranges of illumination which elicits them; the working range of the one process was from 10^{-6} to 10^{4} millilamberts, and that of the other from 10^{-6} to 10^{2}, when tested with a flash of white light lasting 40 msec.

4. Dark-adaptation curves were obtained from the achromats. They showed two parts like a normal dark-adaptation curve. These two parts were accounted for in terms of the two kinds of achromatic process studied above.

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Effect of Hexamethonium on the Augmented Adrenaline Secretion of the Adrenal Gland Causable by Insulin Hypoglycemia

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In view of the blocking effect of hexamethonium on the autonomic ganglia it is deemed desirable to ascertain whether the adrenal medulla is also paralysed by hexamethonium.

The previously reported work[1] provided evidence that the acceleration of adrenaline secretion causable by acetylcholine was definitely suppressed by hexamethonium. Following up these acetylcholine experiments, a further attempt was made in the present investigation to know how hexamethonium affects the augmented adrenaline secretion causable by insulin hypoglycemia.

Some years ago, Schachter[2] demonstrated in dogs that an application of insulin followed by hexamethonium produced more intensive hypoglycemia than that without hexamethonium. From these results it was deduced by him that hexamethonium possessed the paralysing action on the adrenal medulla and suppressed the augmentation of adrenaline secretion causable by insulin hypoglycemia. The evidence presented by him is, however, an indirect one. In the present investigation experiments were made in estimating directly the rate of adrenaline secretion of the adrenal.

Experimental

Methods

Dogs (8.1–14.8 kg. in weight), anesthetized with evipan-sodium, were experimented on. For collecting the adrenal venous blood, the lumbar route preparation of Satake *et al.*[3] was applied. The blood pressure was recorded by a mercury manometer connected with the femoral artery. The blood sugar content was estimated by the method of Hagedorn and Jensen.

Five to 15 minutes before insulin injection, the initial adrenal blood specimens were collected. Then, insulin was injected intravenously in a dose of 7 units per kg. of body weight. Thirty, 60, 70, 80 and 88 minutes after insulin the adrenal venous blood was collected. Ninety minutes after insulin, hexamethonium (Bistrium bromide, Squibb) was given intravenously in a dose of 0.75 mg. per kg. Thereafter the adrenal blood was sampled at intervals. The adrenaline content of the sampled adrenal venous blood specimens was estimated colorimetrically by the Bloor & Bullen's arseno-molybdic acid method,[4] adrenaline (Sankyo Co.) being taken as the standard.

Results and Discussion

The data are shown in table I.

As the control experiments, insulin alone was applied in 3 dogs without injecting hexamethonium.

In Exp. 1, the blood sugar content, which was 0.106–0.107% before insulin, decreased remarkably after insulin. It was determined as 0.072% 30 minutes after insulin application and 0.064% 60 minutes. Thereafter it decreased further and 150 minutes after insulin it reached 0.041%.

The initial adrenaline secretion rate in this dog was too small to be estimated. Thirty minutes after insulin injection the rate of adrenaline secretion was found to have increased to 0.15 μg. per kg. per minute, 30 minutes later it increased further and 0.21 μg. was reached. Then it was measured as 0.24 μg. 70 minutes after insulin, 0.26 μg. 80 minutes, 0.25 μg. 88 minutes, 0.27 μg. 100 minutes, 0.21 μg. 110 minutes, 0.16 μg. 120 minutes and 0.19 μg. 150 minites, respectively.

A similar result was obtained in Exp. 2.

The blood sugar content before insulin was 0.111%. It decreased markedly after insulin and was measured as 0.071% 30 minutes after insulin, 0.066% 60 minutes, 0.064 % 88 minutes, 0.066% 120 minutes and 0.072% 150 minutes.

The adrenaline secretion rate before insulin was immeasurably small. After insulin, the rate of secretion was found to increase and was determined as 0.05 μg. per kg. per minute 30 minutes after injection, 0.07 μg. 60 minutes, 0.09 μg. 80 minutes, 0.10 μg. 88minutes, 0.12 μg. 100, 110 and 120 minutes and 0.03 μg. 150 minutes, respectively.

In Exp. 3, the blood sugar content decreased from 0.106–0.110% and 30 minutes after insulin it reached 0.061%. Thereafter it decreased gradually and was measured as 0.054% 60 minutes after insulin, 0.051% 88 minutes, and 0.049% 115 minutes, respectively.

The initial rate of adrenaline secretion in this dog was 0.04 μg. per kg. per minute. It increased and reached 0.14 μg. 30 minutes after in-

sulin injection. It was measured as 0.10 μg. 60 minutes after insulin, 0.12 μg. 70 minutes, 0.15 μg. 80 minutes, 0.25 μg. 88 minutes, 0.21 μg. 100 minutes and 0.13 μg. 115 minutes, respectively.

The hexamethonium-experiemnts were performed in 4 dogs.

In Exp. 4, the blood sugar content before insulin was 0.119–0.120%. It was reduced by insulin injection and 60–88 minutes after injection it was determined as 0.060–0.064%. Then hexamethonium was given. After hexamethonium blood sugar content increased only slightly.

The initial adrenaline secretion rate was in this experiment 0.02 μg. per kg. per minute. After insulin it increased. It reached 0.13 μg. 60 minutes after insulin and it was measured as 0.17 μg., 0.38 μg. and 0.30 μg. 70, 80 and 88 minutes after insulin, respectively. Ninety minutes after insulin, hexamethonium was injected. Then the rate of adrenaline secretion was found to have decreased remarkably. It was estimated to be 0.08 μg. 5 minutes after hexamethonium, 0.04 μg. 10 minutes, 0.03 μg. 20 minutes and 0.01 μg. 60 minutes.

In Exp. 5, the blood sugar content before insulin was 0.115%. Thirty, 60 and 80 minites after insulin it was determined as 0.063%, 0.058% and 0.054%, respectively. Then, hexamethonium was injected. The blood sugar content was gradually decreased.

Before insulin, the adrenaline secretion rate was 0.01 μg. per kg. per minute. Thirty minutes after insulin it was increased and measured as 0.09 μg. Sixty, 70, 80 and 88 minutes after insulin it was found to be 0.13 μg., 0.28 μg., 0.17 μg. and 0.13 μg., respectively. Hexamethonium was given 90 minutes after insulin. On receiving hexamethonium the rate of secretion was decreased markedly and was measured as 0.02 μg. per kg. per minute 10 minutes after hexamethonium, immeasurably small 20 minutes and 0.006 μg. 50 minutes, respectively.

In Exp. 6, the blood sugar content was reduced from 0.123–0.124% to 0.060% within 30 minutes after insulin. From 60 to 80 minutes after insulin it was 0.054%. Then the injection of hexamethonium was made. The blood sugar decreased further and reached 0.048% 140 minutes after insulin.

The basal rate of adrenaline secretion in this experiment was 0.05–0.06 μg. per kg. per minute. After insulin the rate of secretion was found to increase and was determined to be 0.23 μg. per kg. per minute 30 minutes, 0.21 μg. 60 minutes, 0.23 μg. 70 minutes, 0.22 μg. 80 minutes and 0.25 μg. 88 minutes after insulin. Ninety-five minutes after insulin application, hexamethonium was injected. The rate of secretion then decreased remarkably and it was 0.03 μg. per kg. per minute 10 minutes after hexamethonium, 0.02 μg. 30 and 45 minutes.

In Exp. 7, the blood sugar content decreased from 0.112–0.114% to

TABLE I

The Adrenaline Secretion Rate after Insulin with and without Hexamethonium

Time before or after injection of insulin (min.)	No. of specimen	Quantity of blood (cc.)	Duration of collection (sec.)	Blood flow per kg. per minute (cc.)	Adrenaline content in 1 cc. blood (μg.)	Adrenaline secretion rate per kg. per minute (μg.)	Blood sugar (%)	Blood pressure (mm Hg.)	Heart rate (per min.)	Respiration (per min.)	Body temperature (°C)	Room temperature (°C)
(A) Insulin												
Exp. 1. Dog 14.8 kg. ♀												
before												
15	1	3.8	30	0.51	*		0.107	140	160	23	39.0	14
5	2	4.0	30	0.54	*		0.106	136	136	25	38.9	14
Intravenous injection of insulin (7 units per kg.)												
after												
30	3	6.4	30	0.87	0.17	0.15	0.072	132	156	37	39.0	14.5
60	4	4.1	20	0.83	0.25	0.21	0.064	114	160	40	39.0	14.5
70	5	3.0	20	0.61	0.39	0.24	0·059	106	166	43	39.1	14.5
80	6	3.0	20	0.61	0.42	0.26	0.055	128	170	36	39.1	14.5
88	7	3.1	20	0.63	0.39	0.25	0.054	106	175	40	39.1	14.5
100	8	3.4	20	0.69	0.38	0.27	0.047	110	194	36	38.9	14.5
110	9	3.4	20	0.69	0.30	0.21	0.045	112	198	36	38.9	14.5
120	10	3.4	20	0.69	0.23	0.16	0.045	110	200	36	38.7	14.5
150	11	3.3	20	0.67	0.28	0.19	0.041	106	165	22	38.8	14.5
Exp. 2. Dog 13.3 kg. ♂												
before												
15	1	1.7	30	0.26	*		0.111	138	130	52	38.8	19
5	2	1.7	30	0.26	*		0.111	136	148	58	38.8	19
Intravenous injection of insulin (7 units per kg.)												
after												
30	3	1.9	30	0.29	0.18	0.05	0.071	122	150	70	38.1	19
60	4	1.7	30	0.26	0.27	0.07	0.066	122	146	66	37.9	19
70	5	1.3	30	0.20	0.30	0.06	0.064	116	148	68	37.8	19
80	6	2.1	30	0.32	0.28	0.09	0.064	118	150	52	37.4	19
88	7	2.2	30	0.33	0.31	0.10	0.064	108	142	62	37.5	19
100	8	1.8	30	0.27	0.43	0.12		106	146	56	37.4	19
110	9	1.8	30	0.27	0.46	0.12	0.067	108	150	58	37.5	19
120	10	2.0	30	0.30	0.41	0.12	0.066	104	148	52	37.5	19
150	11	1.6	30	0.24	0.14	0.03	0.072	110	158	48	37.4	19
Exp. 3. Dog 8.1 kg. ♀												
before												
15	1	2.0	30	0.49	0.08	0.04	0.106	116	170	20	38.3	15.5
5	2	1.8	30	0.44	0.09	0.04	0.110	122	158	22	38.1	15.5
Intravenous injection of insulin (7 units per kg.)												
after												
30	3	1.6	30	0.40	0.36	0.14	0.061	110	135	23	37.8	15.5
60	4	2.5	60	0.31	0.33	0.10	0.054	102	132	20	37.8	15.5
70	5	2.2	60	0.27	0.43	0.12	0.053	104	148	22	37.2	15.5
80	6	1.4	30	0.35	0.42	0.15	0.051	104	165	21	37.1	15.5
88	7	1.3	30	0.32	0.77	0.25	0.051	100	160	25	37.0	15.5
100	8	1.3	30	0.32	0.67	0.21	0.049	102	165	24	36.9	15.5
115	9	1.7	60	0.21	0.61	0.13	0.049	98	164	18	36.9	15.5

(B) Insulin-Hexamethonium

Exp. 4. Dog 9.4 kg. ♂

before												
10	1	1.8	30	0.37	0.06	0.02	0.120	154	220	26	37.8	12.5
5	2	1.7	30	0.35	0.06	0.02	0.119	152	185	20	37.7	12.5
		Intravenous injection of insulin (7 units per kg.)										
after												
30	3	1.8	30	0.37	0.24	0.09	0.112	140	203	26	37.5	12
60	4	1.9	30	0.41	0.31	0.13	0.064	132	215	23	37.3	12
70	5	2.0	30	0.43	0.40	0.17	0.060	132	215	25	37.5	12
80	6	2.5	30	0.53	0.72	0.38	0.062	126	200	18	37.5	12
88	7	2.7	30	0.57	0.52	0.30	0.064	130	205	22	37.8	12
90	Intravenous injection of hexamethonium (0.75 mg. per kg.)											
95	8	1.7	30	0.35	0.22	0.08	0.068	90	144	18	37.2	11.5
100	9	1.5	30	0.33	0.11	0.04		92	134	16	36.8	11.5
110	10	1.3	30	0.28	0.09	0.03		78	133	20	36.9	11.5
120	11	1.8	30	0.37	0.13	0.04	0.071	76	138	15	36.5	11.5
150	12	1.7	30	0.35	0.03	0.01	0.075	80	140	17	35.9	11.5

Exp. 5. Dog 9.5 kg. ♂

before												
15	1	1.4	30	0.29	0.05	0.01	0.115	134	145	33	37.6	12.5
5	2	1.4	30	0.29	*		0.115	134	145	35	37.6	12.5
		Intravenous injection of insulin (7 units per kg.)										
after												
30	3	1.7	30	0.36	0.26	0.09	0.063	134	122	56	37.1	12.5
60	4	1.8	30	0.38	0.33	0.13	0.058	128	135	68	37.2	12.5
70	5	1.9	30	0.40	0.71	0.28		132	126	60	37.2	12.5
80	6	1.7	30	0.36	0.47	0.17	0.054	104	133	72	37.1	12.5
88	7	2.0	30	0.42	0.32	0.13		102	135	60		
90	Intravenous injection of hexamethonium (0.75 mg. per kg.)											
100	8	2.0	60	0.21	0.11	0.02	0.051	74	86	40	37.0	12
110	9	1.7	60	0.18	*		0.041	64	80	24	36.8	12
140	10	1.0	90	0.07	0.08	0.006	0.039	94	111	7	35.6	12

Exp. 6. Dog 10.1 kg. ♂

before												
15	1	2.3	30	0.46	0.11	0.05	0.123	140	185	37	38.9	12
5	2	2.2	30	0.44	0.13	0.06	0.124	140	192	40	38.8	12
		Intravenous injection of insulin (7 units per kg.)										
after												
30	3	2.1	30	0.42	0.54	0.23	0.060	124	132	59	39.0	12
60	4	1.8	30	0.36	0.59	0.21	0.054	132	160	61	38.9	12
70	5	1.8	30	0.36	0.64	0.23	0.054	124	175	66	38.8	11.5
80	6	1.5	30	0.30	0.74	0.22	0.054	118	165	60	38.8	11.5
88	7	1.6	30	0.32	0.79	0.25	0.053	120	148	42		
95	Intravenous injection of hexamethonium (0.75 mg. per kg.)											
105	8	1.4	60	0.14	0.24	0.03	0.047	88	130	33	38.5	12
125	9	1.8	60	0.18	0.13	0.02	0.047	104	136	40	37.7	12
140	10	1.5	60	0.15	0.16	0.02	0.048	100	135	26	37.4	12

Exp. 7. Dog 14.0 kg. ♂

before												
15	1	1.8	60	0.13	0.16	0.02	0.112	146	165	14	38.5	14.5
5	2	1.9	60	0.14	0.15	0.02	0.114	120	188	16	38.2	14.5
		Intravenous injection of insulin (7 units per kg.)										
after												
30	3	1.5	60	0.11	0.31	0.03	0.066	112	180	20	38.1	14
60	4	1.2	60	0.09	0.66	0.06	0.051	94	163	27	38.0	14
70	5	1.2	60	0.09	0.60	0.05	0.047	94	170	29	37.9	14
80	6	1.8	60	0.13	0.64	0.08	0.045	98	175	28	37.8	14
88	7	1.4	60	0.10	0.68	0.07		94	165	30		14
90	Intravenous injection of hexamethonium (0.75 mg. per kg.)											
100	8	1.5	60	0.11	0.12	0.01	0.039	70	121	20	37.1	14
110	9	1.8	90	0.09	0.22	0.02	0.035	72	115	20	36.9	14
120	10	1.3	60	0.09	0.22	0.02	0.035	74	120	20	36.7	14
150	11	1.9	60	0.14	0.15	0.02	0.030	82	128	8	36.1	14

* Less than 0.05 μg.

0.066% 30 minutes after insulin and it was 0.045% 80 minutes, 0.039% 100 minutes, 0.035% 120 minutes and 0.030% 150 minutes after insulin.

In this experiment, the initial adrenaline secretion rate was 0.02 μg. per kg. per minute. On receiving insulin, the rate of secretion was measured as 0.03 μg. 30 minutes after insulin, 0.06 μg. 60 minutes, 0.05 μg. 70 minutes, 0.08 μg. 80 minutes and 0.07 μg. 88 minutes, respectively. Hexamethonium was then injected. The secretion rate of adrenaline was decreased and was measured as 0.01 μg. per kg. per minute 10 minutes after hexamethonium and 0.02 μg. 20, 30 and 60 minutes.

Comparing the results obtained in the hexamethonium-experiments mentioned above with those of the control ones, it is evident that the augmented adrenaline secretion causable by insulin hypoglycemia is suppressed by the application of hexamethonium.

In my previous investigation it was proved that the augmentation of adrenaline secretion causable by acetylcholine was inhibited by hexamethonium. Since acetylcholine acts exclusively peripherally on the adrenal medulla to accelerate the adrenaline secretion, it is sure that in this case the site of action of hexamethonium is the adrenal medulla itself.

Therefore it is reasonable to suppose that in the case of insulin hypoglycemia, which acts through the central mechanism to augment the adrenaline secretion in releasing acetylcholine at the terminals of adrenalinosecretory nerve fibres, hexamethonium suppresses the augmentation of adrenaline secretion also by acting directly on the adrenal medulla.

Summary

In dogs anesthetized with evipan-sodium, the adrenal venous blood specimens were collected through the lumbar route preparation. The adrenaline content of the specimens was measured colorimetrically by means of the Bloor & Bullen's arseno-molybdic acid method. The blood sugar content was also determined simultaneously.

Insulin was applied intravenously in a dose of 7 units per kg. of body weight. The adrenaline secretion rate was increased gradually, and from 1 to 2 hours after insulin a definite increase was invariably observed. At the height of augmentation of adrenaline secretion, hexamethonium was injected intravenously in a dose of 0.75 mg. per kg. In all cases the secretion rates were cut down to low levels by hexamethonium. It was measured in 3 cases as 0.02–0.04 μg. per kg. per minute after hexamethonium against 0.13–0.30 μg. before hexamethonium. In one case, it was 0.01 μg. against 0.07 μg.

Thus it is concluded that hexamethonium acts to inhibit the accelerating adrenaline secretion causable by insulin hypoglycemia.

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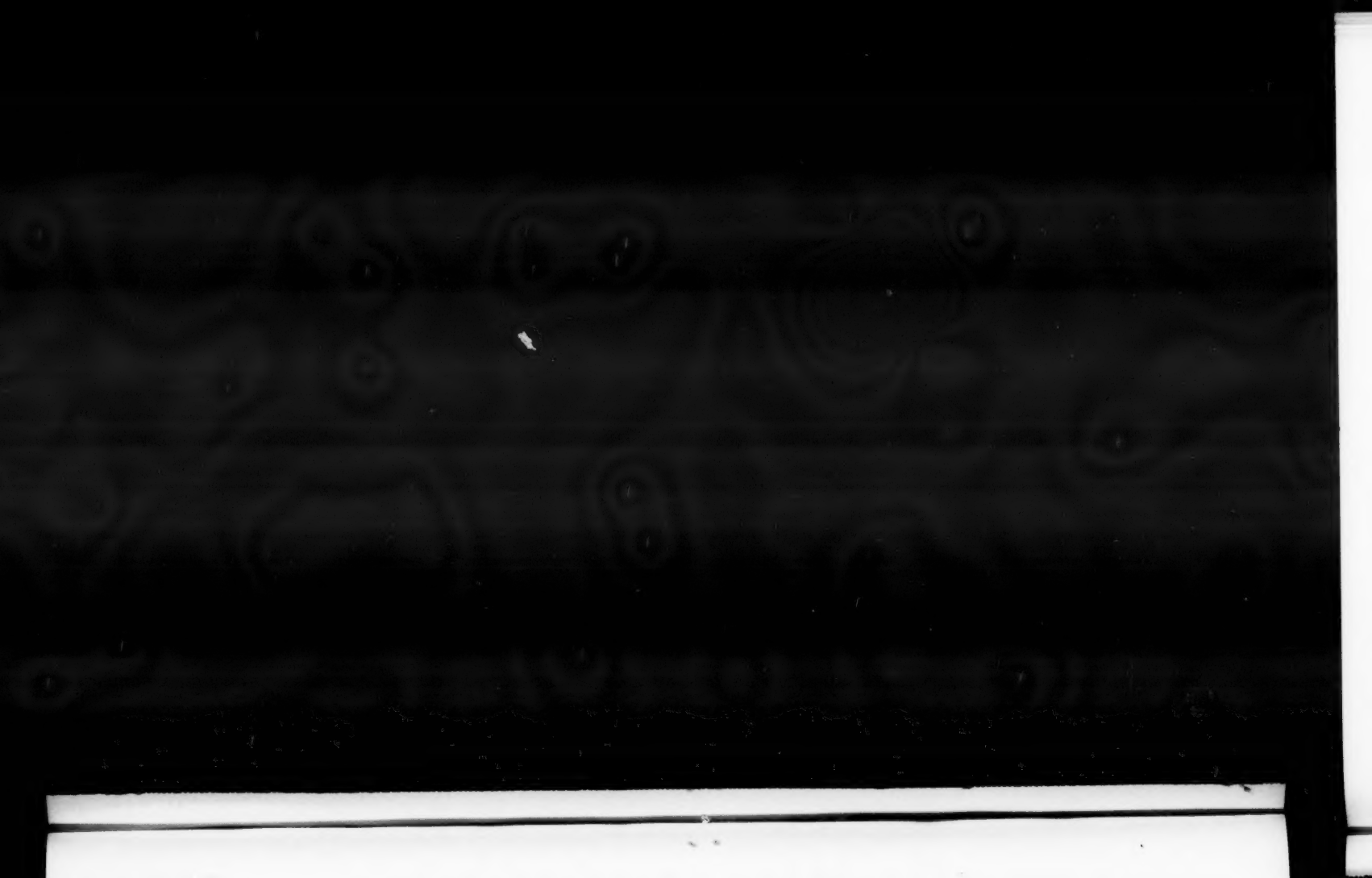
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